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THE UNIVERSITY OF ALBERTA

DIETARY LIPID MODULATION OF CARDIAC FUNCTION IN THE RAT

BY

© NAJI H. TAWFIK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA
SPRING 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Dietary Lipid Modulation of Cardiac Function in the Rat submitted by Naji H. Tawfik in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmacology.

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External Examiner

Date: OCT 28 1986

TO MY PARENTS, AFAF AND DAHLIA

ABSTRACT

The effects of prior dietary lipid manipulation on the function of isolated rat heart tissues has been investigated. Isocaloric diets providing 28-34 en% as fat were fed to young adult male rats for 9-12 week periods. The diets provided adequate protein, vitamins and essential fatty acids but provided predominantly one class of fatty acid, namely saturated acids (16:0 & 18:0; sheep perirenal fat; SKF-group), oleic acid (18:1,ω9; canola variety, rapeseed oil; CAN-group), linoleic acid (18:2,ω6; sunflower oil; SFO-group) and linolenic acid (18:3,ω3; linseed oil; LIN-group).

Diets had no effect on body weight gain, heart rate or blood pressure. Tissues from the SKF-group had the greatest twitch tension while tissues from the CAN-group were consistently low when subjected to a calcium concentration-effect assay. Atria from SFO- and LIN-groups behaved as the CAN-group, while corresponding papillary muscles behaved as the SKF-group. Differences in contractility were abolished by 0.01 mM indomethacin or by chemical skinning. The inotropic actions of BAY K8644 was greatest in CAN atria and least in SKF atria. Only SKF papillary muscles responded to this drug.

Ca^{2+} uptake into SR vesicles was elevated in CAN, LIN and SFO atria, and in LIN, SFO and SKF ventricles. Ca^{2+} dependent ATPase activity was elevated in SKF, CAN and SFO atrial SR but not in ventricular SR.

Threshold to dysrhythmia induced by $[Ca]_o$ or isoprenaline was greatest in CAN tissues. Differences in threshold to dysrhythmia were abolished in atria, but not papillary muscles by 0.01 mM indomethacin,

20 mM mannitol or 1 μM allopurinol.

Analysis of membrane phospholipids indicate a high degree of homeostasis in Σsaturated fatty acid, Σarachidonate and unsaturation index.

Maintaining a constant membrane fluidity with available acyl CoA esters is concluded to result in changes in the heart's ability to produce prostaglandins, and their by-products free radicals.

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1. INTRODUCTION

Lipids form a group of compounds with extremely varied chemical nature. Fatty acids are the main consituents of food fats and oils as well as of depot fat in man and animals. They are composed of carbon, hydrogen and oxygen. The carbon atoms form a chain varying in length from 4 to 26 carbons. At one end of the chain a carbon atom forms a methyl group, and at the other end the carbon forms part of a carboxyl group. If the carbon atoms which form the body of the chain have their two remaining valencies occupied by hydrogen atoms, the molecule is a saturated fatty acid. If, on the other hand, one hydrogen atom is missing from each of two neighbouring carbons, a double bond is formed between these two carbon atoms and the fatty acid is said to be monounsaturated. The fatty acid is polyunsaturated if two or more double bonds are formed in the carbon chain.

The older nomenclature for fatty acids reflected natural origin of many acids, but gave little indication of structure. A more systematic descriptive system has been introduced based upon chain length and degrees of unsaturation (IUPAC-IUB Commission on Biochemical Nomenclature, 1976). For example, palmitic acid, a saturated fatty acid with a chain length of 16 carbons, is designated by the symbol C16:0.

The terminology for monounsaturated and polyunsaturated fatty acids takes into consideration, not only chain length and the number of enoic bonds present, but also their respective positions on the chain. The positions of double bonds are indicated by the symbol Δ, ω or n followed by one or more numbers giving their position on the carbon chain.

Carbon atoms may be numbered from the carboxyl (Δ nomenclature) or from the methyl (ω or n nomenclature) group. Linoleic acid can be represented as C18:2Δ9,12 indicating two double bonds, the first one located on the 9th carbon, counting from the carboxyl group and the second one on the 12th carbon. The formula C18:2,ω6 could also designate linoleic acid where the first carbon bearing a double bond is number 6 counting from the methyl group. In this thesis the latter terminology is adopted, and the symbol Δ is used only to indicate the site of action of desaturase enzymes.

1.1 DIETARY FAT

Fat constitutes as much as 40-50% of total caloric intake for Northern Americans (Field and Clandinin, 1984) and is composed of a variety of different types of compounds varying in physical properties. Dietary fats contain practically no free fatty acids. Instead these are present in the form of triacylglycerols consisting of one molecule of glycerol and three molecules of fatty acid at α, β, and α' positions.

The fatty acid distribution on the glycerol molecules in nature seems not to occur by chance. It is now known that, for certain types of fats there is a preference for certain fatty acids to occupy positions α, β or α'. In cow's milk butyric acid (C4:0) is found mainly in the α' position and stearic acid (C18:0) in the α position (Breckenridge, 1978). In human milk, unsaturated acids occupy mainly the α and α' positions (Breckenridge et al., 1969). The position of the particular fatty acids in triacylglycerols is of significance during the digestion and absorption of fats when monoglycerides and diglycerides are formed. It is of importance also during the formation of phospholipids in the body, where enzyme activity may be specific for the different positions on the glycerol molecule. This will be discussed later.

Fats of animal origin have a high percentage of saturated fatty acids (butter contains about 50%) while vegetable particularly corn, soybean and sunflower seed oils, contains less than 15% saturated fatty acids and more than 55% polyunsaturated fatty acids (Sheppard et al., 1978). A recent report by Block et al. (1985) identified beef as the most important single contributor of fat in the US diet; it being the source of 18-20% of total saturated fat consumed by the average individual. The same report showed a decline in the consumption of the more expected sources of fat, such as butter (2.4% of total fat), and that was accompanied with an increased consumption of margarine (4.5% of total fat).

Although technical problems surround the measurement of dietary intake, there is strong evidence that indicates a change in the North American diet between the late 1950s and the mid 1970s. Food consumption data provided by Friend et al. (1978) showed a decline in the purchase of whole milk and cream by almost 25% and the use of butter by over one third. The per capita consumption of eggs has dropped by approximately 15% during this period. Of major note is that consumption of vegetable fats has increased by almost 75%.

1.2 FATTY ACIDS: BIOCHEMISTRY AND METABOLISM

1.2.1 Fatty Acid Biosynthesis

The existence of a sensitive and multifaceted control system for fatty acid synthesis has been recognized for many years. Fatty acids are stored as neutral lipids in the form of triacylglycerols. A balance is expected to be maintained between (i) the storage of fatty acids as triacylglycerols and (ii) the synthesis of phospholipids as essential membrane components. The fatty acids required for these lipids can be derived either from a dietary source or from de novo synthesis. Saturated and ω-9 monounsaturated fatty acids can be derived from either source but polyunsaturated fatty acids (ω-3 & ω-6) if not consumed directly can only be derived from dietary linoleic (C18:2,ω6) or α-linolenic acids (C18:3,ω3) (Jeffcoat and James, 1984) which in turn have been derived directly or indirectly from a plant source. Since these fatty acids can not be synthesised by animal tissue they are referred to as "essential fatty acids". The chronic deficiencies of these fatty acids from the diet lead to a long list of symptoms (Alfen-Slaten and Aftergood, 1968; Holman, 1968; Holman, 1970; Soderhjelm et al., 1970) and the clinical manifestations range from parakeratosis to reduced growth rate and increased susceptibility to bacterial infection (Van Dorp, 1971). Other effects reported were reduced myocardial contractility (Ten Hoor et al., 1973) and abnormal platelet aggregation (Hornstra, 1974).

In most organisms, the primary product of fatty acid biosynthesis is palmitic acid (C16:0). It is from this common precursor that most other fatty acids found in membranes are formed by elongation and desaturation (Thompson and Martin, 1984). Since palmitate itself is a major membrane fatty acid species, under certain conditions a cell could theoretically modify its fatty acid composition by adjusting the fate of further modification.

The basic reactions of fatty acid biosynthesis are the same in all organisms. The initial biosynthetic step is a conversion of acetyl Co A to malonyl-Co A by the enzyme acetyl-Co A carboxylase (Numa and Tanabe, 1984). Two carbon segments of malonyl-Co A molecules are then sequentially condensed together in a multistep process catalyzed by the fatty acid synthetase systems (Alberts and Greenspan, 1984).

1.2.2 Fatty Acid Modification Mechanisms

1.2.2.1 Fatty acid elongation

Most cells contain a significant proportion of fatty acids which have 18 or more carbon atoms, with the longer chains being major components of certain specialized tissues, e.g., myelin of mammalian brain contains high level of 22- and 24- carbon components (Thompson, 1980).

There are two enzymatic systems which carry out elongation in eukaryotic cells. A system associated with mitochondria involves reversing some of the reactions of the β-oxidation pathway. Thus, β-oxidation and elongation both make use of acetyl-Co A acetyltransferase, 3 hydroxyacyl-Co A dehydrogenase, and enoyl-Co A hydratase with the difference being in the final step of fatty acid elongation which utilizes an enzyme, enoyl-Co A reductase, which is not a part of the β-oxidation system (Hinsch and Seubert, 1975). The physiological significance of this particular pathway in controlling cellular fatty acid composition is questionable, since it exhibits its highest activity toward 8 or 10 carbon Co A derivatives and appears to be inhibited by stearoyl- and palmitoyl-Co A (Hinsch *et al.*, 1976).

Although few details are known concerning the enzyme systems for microsomal fatty acid elongation, it appears that this may be the most important source of longer chain (C18 to C24) fatty acids in many tissues (Thompson, 1980). The substrate for the chain-lengthening reactions was shown by Nugteren (1965) to be the acyl-Co A derivatives. The pathway starts with the condensation of acyl-Co A and malonyl-Co A, followed by a reduction, dehydration and a second reduction to yield a chain longer by two carbons (Jeffcoat and James, 1984). Substrate specificity varies markedly from one cell type to another. Studies on rat brain indicated that the preferred substrates were γ-linolenic (C18:3,ω-6) (100%) > palmitic (C16:0) (75%) > arachidonic (C20:4,ω6) (57%) > α-linolenic (C18:3,ω-3) (30%) > linoleic (C18:2,ω6) (10%) (Cook, 1982).

1.2.2.2 Fatty acid desaturation

The introduction of double bonds into the hydrocarbon chain of a fatty acid is considered as one of the important means of regulating membrane fluidity (Stubbs and Smith, 1984). The reaction involves the dehydrogenation of two adjacent carbons in the fatty acyl chain with a high degree of positional specificity. Although recent studies have been focussed on mammalian cells, early studies were carried out using Euglena gracilis (Nagai and Block, 1968). Extracts of this organism were able to convert stearic acid into oleic acid (C18:1,ω9) by a reaction that required both reduced pyridine nucleotide and molecular oxygen. Subsequent fractionation of these extracts resulted in the first evidence that the desaturase was not a single enzyme but was composed of at least 3 individual proteins: an NADPH oxidase, a non haem iron sulphur protein, ferredoxin, and the desaturase. Further studies demonstrated that incorporation of saturated fatty acids into triacylglycerols prevented the desaturation, and established that the true substrates for mammalian desaturates were the coenzyme-A esters (Raju and Reiser, 1967; Strittmatter et al., 1974). The only exception is the observation of Pugh and Kates (1977) that the Δ^5-desaturase of rat liver can act both on the coenzyme-A or phospholipid form of eicosatrienoic acid (C20:3,ω6) to form arachidonic acid.

The desaturation of saturated fatty acids to form monoenoic acids is catalyzed by Δ^9-desaturase (Jeffcoat, 1977), and it is strongly believed that stearoyl-Co A is the preferred substrate for this enzyme (Morris, 1970; James et al., 1977).

In contrast to the Δ^9-desaturase, the Δ^6- and Δ^5 desaturases (and may be the Δ^4-desaturase) are responsible for the conversion of essential polyunsaturated fatty acids of the ω-3 and ω-6 series into more unsaturated acids (Jeffcoat and James, 1984). These workers summarized the types of reaction as follows:

$$\begin{tikzcd}
\begin{array}{c}\text{Linoleic acid}\\(C18{:}2,\omega 6)\end{array} \arrow[r, "\Delta^6"] & \begin{array}{c}\gamma - \text{Linolenic acid}\\(C18{:}3,\omega 6)\end{array} \arrow[r, "\text{Elongase}"] & \begin{array}{c}\text{Eicosatrienoic acid}\\(C20{:}3,\omega 6)\end{array} \arrow[d, "\Delta^5\ \text{desaturase}"] \\
 & & \begin{array}{c}\text{Arachidonic acid}\\(C20{:}4,\omega 6)\end{array}
\end{tikzcd}$$

$$\begin{tikzcd}
\begin{array}{c}\alpha - \text{Linolenic acid}\\(C18{:}3,\omega 3)\end{array} \arrow[r, "\Delta^6", "\text{desaturase}"'] & C18{:}4,\omega 3 \arrow[r, "\text{Elongase}"] & C20{:}4,\omega 3 \arrow[r, "\Delta^5", "\text{desaturase}"'] & C20{:}5,\omega 3
\end{tikzcd}$$

The Δ^6-desaturase acts on both linoleic acid and α-linolenic acid, although the latter was found, to be the preferred substrate (Brenner and Peluffo, 1966). Therefore when both acids are present there is competition for the two substrates. In the Western Diet the principal substrate is linoleic acid (C18:2,ω6) (Philipson et al., 1985), and therefore arachidonic acid is formed in substantial amounts.

Jeffcoat and James suggested that under those circumstances where neither linoleic nor α-linolenic acids are adequately present in the diet (essential fatty acid deficiency), then the substrate for Δ^6-desaturase is oleic acid:

$$\underset{(\text{C}18{:}0)}{\text{Stearic acid}} \xrightarrow[\text{desaturase}]{\Delta^9} \underset{(\text{C}18{:}1\omega 9)}{\text{Oleic acid}} \xrightarrow[\text{desaturase}]{\Delta^6} \text{C}18{:}2,\omega 9 \xrightarrow{\text{Elongase}} \text{C}20{:}2,\omega 9 \xrightarrow[\text{desaturase}]{\Delta^5} \text{C}20{:}3,\omega 9$$

It is now generally believed that there exists a Δ^8-desaturase in the rat and human testes (Albert and Coniglio, 1977; Albert et al., 1979):

$$\text{C}18{:}2,\omega 6 \xrightarrow{\text{Elongase}} \text{C}20{:}2,\omega 6 \xrightarrow[\text{desaturase}]{\Delta^8} \text{C}20{:}3,\omega 6 \xrightarrow[\text{desaturase}]{\Delta^5} \text{C}20{:}4,\omega 6$$

The existence, however, of Δ^4 desaturase remains controversial (Budny and Sprecher, 1971; Prasad and Joshi, 1979; Slack et al., 1979).

1.2.2.3 Trans - fatty acids

Partial hydrogenation of vegetable oils modifies the chemical and physical properties of their fatty acids with the transformation of the naturally produced cis-unsaturated fatty acids into trans-isomers. These new types of fatty acids are present in the different varieties of margarines and shortenings in use. Trans-fatty acids adopt a linear configuration in contrast to the curved configuration of the cis-isomers. Such fatty acids have been discovered in a number of plant species, mainly in seed fats but also in leaves. Oil seeds used for vegetable fat production were not found to contain trans-isomers (Sommerfeld, 1983). There have been two reports that some varieties of rapeseed oil contain brassidic acid (trans-erucic acid, C22:1,ω9) (Hartman, et al., Turchetto and Lorusso, 1977), although this has also been denied (Fogerty et al., 1978). On the other hand, trans-unsaturated fatty acids are found in animal fats. In most animals, they originate from the diet, although in ruminants and marsupials these fatty acids are synthesized in the rumen or rumen-like stomach by microbial hydrogenation of polyunsaturated fatty acids (Niehaus, 1978).

One aspect of trans-fatty acids that has received attention is their influence on polyunsaturated fatty acid metabolism, in particular the ability of trans-trans-C18:2,ω6 to inhibit the elongation and desaturation of linoleic acid (Kurata and Privett, 1980). Like the trans-trans-dienoic acids, trans-monoenoic acids will inhibit the conversion of linoleic to arachidonic acid (Mahfouz et al., 1980) with a subsequent effect on eicosanoid production.

The rate of metabolism of trans- fatty acids via the β-oxidation pathway was slower in mitochondria isolated from rat cardiac muscle (Lawson and Kummerow, 1979), and so the availability of only trans rather than cis-isomers during periods of increased demand for energy is likely to be a limiting factor with serious consequences (Kummerow, 1979).

1.2.3 Oxidation of Fatty Acids

1.2.3.1 β-oxidation

Under normal aerobic conditions, the energy requirements of the heart are met preferentially by oxidation of free fatty acids (Katz and Messineo, 1983) which are trapped in cells in the form of fatty (acyl) esters containing coenzyme A (acyl-Co A) or carnitine (acyl-carnitine) (Groot et al., 1976). The acyl-Co A may be either incorporated into triacylglycerols while in the cytosol or broken down to form acetyl-Co A by β-oxidation after transport into the mitochondria. Normally, oxidation predominates with the formation of the two carbon fragment acetyl Co A (Bremer and Osmundsen, 1984), which is readily incorporated into the citric acid cycle and oxidized to carbon dioxide and water.

1.2.3.2 Lipid peroxidation

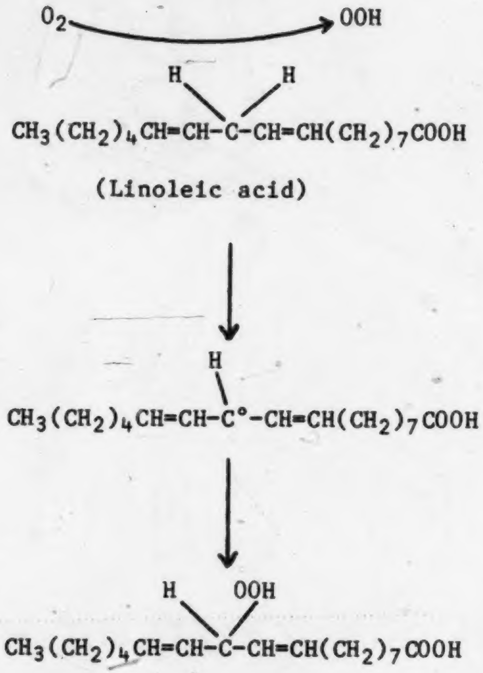
The generation of free radicals is virtually ubiquitous in cells (Thompson, 1984), and there is accumulating evidence from both plant and animal systems that harmful free radical reactions contribute to aging (Harman, 1981). Free radicals can be formed enzymatically by mitochondrial respiration (Nohl *et al.*, 1978), the enzyme xanthine oxidase (Harman, 1981), as well as through nonenzymatic reactions of oxygen with organic compounds (Mead, 1976). In enzymatic reactions involving oxygen, the superoxide anion (O_2^-) is the radical species formed, and it spontaneously dismutates to give H_2O_2 and oxygen as follows:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Hydrogen peroxide and superoxide then in turn react to form the hydroxyl radical (OH°):

$$O_2^- + H_2O_2 \rightarrow OH^- + OH^\circ + O_2$$

It is known that polyunsaturated fatty acids such as linoleic acid (C18:2,ω6) easily undergo peroxidation giving rise to free radicals, hydroperoxides, endoperoxides and other products (Thompson, 1980, Brisson, 1981):


$O_2 \rightarrow OOH$

$CH_3(CH_2)_4CH{=}CH{-}\overset{H\ \ H}{C}{-}CH{=}CH(CH_2)_7COOH$

(Linoleic acid)

$\downarrow$

$CH_3(CH_2)_4CH{=}CH{-}\overset{H}{C}^{\circ}{-}CH{=}CH(CH_2)_7COOH$

$\downarrow$

$CH_3(CH_2)_4CH{=}CH{-}\overset{H\ \ OOH}{C}{-}CH{=}CH(CH_2)_7COOH$

Hydroperoxide

6

Such products could cause damage to biomembranes and the intracellular organelles, and the nature of this damage may be varied (Tappel, 1973; Oster, 1980). In experiments done on plant cells free radicals stimulated the deacylation of membrane phospholipids, and promoted a phase separation within the bilayer resulting in a mixture of liquid-crystalline and gel phases (Pauls and Thompson, 1980).

Under normal conditions, the cells are protected against free radicals and other harmful peroxidation compounds by natural antioxidants such as vitamin E, and enzymes such as superoxide dismutase, catalase and peroxidase (Thompson, 1984).

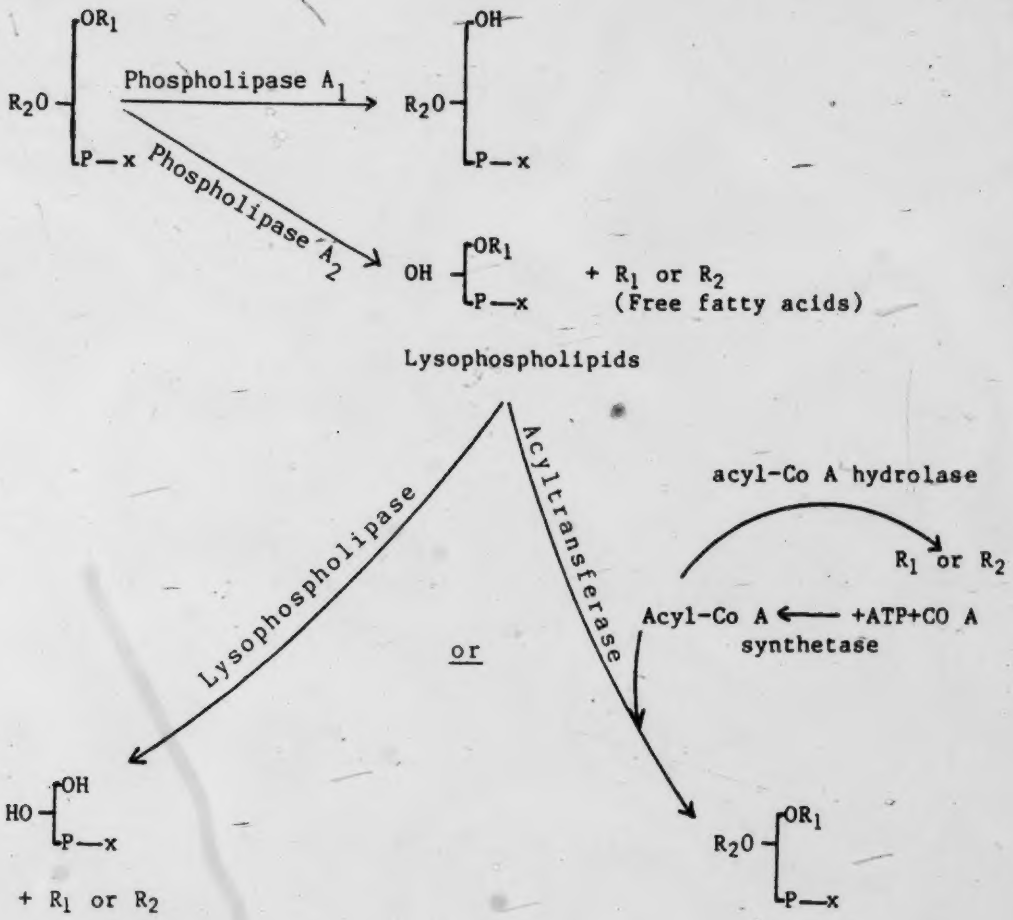
1.2.4 Fatty Acyl Chain Positioning in Phospholipids

Phospholipids are diesters of phosphoric acid, and constitute an important group of lipidic compounds. A great proportion of the phospholipids found in animals and in plants belong to the family of phosphoglycerides. These compounds are formed by the esterification of diacylglycerol with phosphoric acid, and their classification is based on the particular residues linked to the phosphoric acid molecule. They are called phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. Glycerophospholipids constitute the major lipid component of membranes. A given membrane type contains different phospholipid classes in fixed proportion, while another membrane in the same cell might be quite different in its phospholipid distribution (White, 1973).

There is widely held agreement that the enzymes catalyzing *de novo* synthesis of glycerophospholipids from fatty acids and water soluble precursors are partly responsible for the nonrandom placement of fatty acids in the final products (Thompson and Martin, 1984). This view is supported by the fact that all phospholipid precursors (starting with phosphatidic acid) do contain mainly saturated fatty acids in their α-position and unsaturated fatty acids in their β-position (Brisson, 1981). The mechanism of this specific positioning has not been explained. Bell and Coleman (1980) studied the enzymes that catalyze the addition of the first acyl group to glycerol-3-phosphate. Their work indicated that the relative activities of the microsomal and mitochondrial enzymes vary from tissue to tissue and also during cell differentiation. The two enzymes differ in a number of ways, including their specificity for fatty acyl-Co A (Halder *et al.*, 1979). The mitochondrial acetyltransferase was shown to prefer saturated acyl chains, while that associated with microsomes showed little selectivity between palmitoyl-Co A and oleoyl-Co A. The addition of the second fatty acyl group to lysophosphatidic acid is carried out by another distinct microsomal acyltransferase enzyme, which has a preference to place unsaturated fatty acids in the β-position (Bell and Coleman, 1980).

It is still uncertain whether these or other enzymes exhibit an *in vivo* preference for substrates having a specific acyl chain composition. Also, the relative importance of *de novo* synthesis vs. deacylation - reacylation in determining the final steady-state phospholipid acyl chain distribution remains unclear.

The relation between acyl chain turnover and phospholipid biosynthesis is summarized (Irvine, 1982) as follows:



P = Phosphate group
x = Base (e.g. choline)

R_1 and R_2 = Fatty acyl chains

If one considers the striking differences in fatty acid composition among phospholipid classes from the same membrane, it becomes easy to realize that phospholipid headgroups influence the specificity of acyltransferases during reacylation (Thompson and Martin, 1984). Recent investigations indicated a competition among different phospholipid classes for certain fatty acids. Murine leukemia cells were found to incorporate ^{14}C-arachidonic acid initially into phosphatidylcholine (Kannagi et al., 1982). The arachidonate, with time, was gradually transferred to phosphatidylethanolamine, most probably through acylation-deacylation. This time-dependent selectivity during reacylation may be explicable by the finding of Okuyama et al. (1975), that acyltransferase selectivity is determined in part by the concentration of the lysophospholipid acceptor.

1.3 STRUCTURE AND FUNCTION OF CELL MEMBRANES

Over the first four decades of this century, substantial amounts of qualitative evidence indicated that surface layers of the cell must be predominantly lipid. This arose partly from the permeability studies of such investigators as Overton (1895), and partly from the provocative evidence obtained through the experiments of Gorter and Grendel (1925). It began with the postulation that membranes were, in part, lipoidal in character, since hydrophobic molecules were known to penetrate membranes more easily than hydrophilic ones. However, there was much doubt as to whether the membrane was a homogenous lipid layer, or a mosaic of different components with the possibility of containing substantial pores. In 1935 Danielli and Harvey were able to show that protein adsorbed strongly to lipid surfaces even when these lipid surfaces were of low surface free energy. These observations led to the concept that proteins might be an associated part of cell membranes. Danielli and Davson (1935) were among the first to contemplate protein as a membrane component. They proposed a model in which the phospholipid molecules were arranged in such a way that fatty acyl chains were directed towards the inside of the bilayer, while the polar head groups to the outside to which globular proteins were attached. This model was later modified to include proteins extending through the thickness of the membrane (Davson and Danielli, 1943). With the advent of electron microscopy it was possible to recognize the ultrastructural features of membranes (Robertson, 1959). Most of these studies were of membranes of myelin but the membrane model proposed, usually referred to as the unit-membrane hypothesis, was suggested as the basic structure of all cell membranes.

1.3.1 The Fluid-Mosaic Concept of Membrane Structure

The fluid mosaic model has evolved by a series of stages from an earlier version (Lenard and Singer, 1966; Wallach and Zahler, 1966; Glaser et al., 1970; Singer, 1971). According to this theory the matrix of cell membranes is a bilayer of phospholipids to which two categories of protein are bound, either adsorbed by predominantly polar forces (peripheral proteins) or interpolated into the bilayer in direct contact with the hydrophobic region of the membrane (integral proteins). The structure is thought to be fluid in the sense that individual molecules are able to diffuse readily in the plane of the membrane to form regions of distinctive affinity or character over the membrane surface. At least two parameters are believed to participate in determining membrane fluidity: the rate of different motions of molecular elements, and ordering of the hydrocarbon chains (Brenner, 1984).

1.3.2 The Topogenesis of Lipids in Membranes

The term "lipid topogenesis" was used by Bell et al. (1981) as a categorial term for those intracellular processes occurring simultaneously with or shortly after the synthesis of complex lipids. The early events of lipid topogenesis include lipid synthesis, the integration of lipids into membranes, and lipid translocation across membranes. Later events involve lipid movement to other membranes and structures, the sorting of different complex lipids from each other to assemble structures with distinct lipid compositions, and the formation and maintenance of lipid asymmetry within biological membranes and other structures.

In addition to glycerophospholipids, membranes contain other varieties of lipidic compounds. Sphingolipids constitute one group of compounds that resembles glycerolipids except that a long-chain amino alcohol (sphingosine) replaces the glycerol in the pivotal position. Sphingomyelin has a fatty acid amide bonded to the amino group of sphingosine as well as choline in phosphodiester linkage to the terminal hydroxyl group (Quinn, 1976). The content of sphingomyelin varies considerably in membranes from diverse sources, but in many systems, the sum of the two choline-containing lipids, sphingomyelin and phosphatidylcholine, constitutes about half of the total phospholipid although the molar ratio of these two respective components varies considerably (Barenholz and Thompson, 1980). In most organs, the ratio of phosphatidylcholine to sphingomyelin is fairly constant in the same organ of different mammalian species (Barenholz, 1984). This is not true for brain, an organ rich in sphingomyelin, where large variations in this ratio between various species are found (Rouser et al., 1972).

Ceramides constitute a class of sphingolipids that lack phosphoric acid and choline. Esterification of sugars through the terminal hydroxyl group of sphingosine gives rise to the separate class of glycolipids (Brisson, 1981). These sugars can be either simple in structure, possessing only a single galoctosyl residue (cerebrosides), or complex oligosaccharides containing neutral and charged amino sugars (gangliosides). The plasmalemma contains most of the cellular glycolipids: these lipids are not usually found in mitochondria, endoplasmic reticulum or nuclear membranes (Quinn, 1977). Cholesterol is an important component of membranes in all mammalian cells (Cooper and Strauss, 1984), and its role will be discussed later. Mitochondrial membranes are characterized by the presence of cardiolipin (diphosphatidylglycerol-DPG) which is confined almost entirely to this organelle and is located predominantly in the inner membrane (Green et al., 1982).

It is still unclear the way by which these complex lipids are integrated into the different membranes of the cell, and little information exists on the topography of complex lipid synthesis in organelles such as Golgi, mitochondria and peroxisomes.

1.3.3 Membrane Fluidity

In the past decade the functional importance of membranes in many biological processes has been widely recognized. Many enzymes, receptors and transport proteins are situated in membranes and are under potential influence of the physical state of the membrane lipids. It is then obvious that the phospholipid bilayer provides more than a barrier to the free flow of ions and solutes in and out of the cell. Most membrane enzymes studied require the lipids to be in a "fluid" state for optimum activity (Stubbs, 1983; Spector and Yorek, 1985). The term fluidity is largely used in membrane literature and is sometimes confusing. The term fluid refers to the physical state of the phospholipid acyl chains when the temperature is above the gel-liquid crystalline phase transition temperature (Melchior and Steim, 1976). Below the transition temperature (T_c) the fatty acyl chains are packed together in an ordered, crystalline form; above T_c the chains become more disordered due to the flexing of the chain at the higher temperature (Shinitzky, 1984). The temperature at which the phase transition occurs is characteristic of the fatty acyl chain and phospholipid head group, and is also affected by other membrane components such as cholesterol and protein (Rittenhouse, _et al_., 1974;

Huang and Mason, 1982; Stubbs, 1983; Brenner, 1984; Shinitzky, 1984).

1.3.3.1 Effect of fatty acyl chains

Fatty acyl modifications can influence the degree of ordering in the hydrocarbon core of the lipid bilayer. Components directly affecting the physical properties of the acyl chains are the unsaturation and acyl chain length (Stubbs and Smith, 1984). Effects on membrane structure can arise both from changes in the esterified fatty acids and from interaction with non-esterified fatty acids (Stubbs, 1983). Phospholipids containing fully saturated or *trans*-unsaturated acyl chains can pack together tightly (Seelig and Waespe-Sercevic, 1978; Brenner, 1984). On the other hand, the introduction of a *cis*-double bond into a phospholipid acyl chain, resulting in a rigid bend in the otherwise flexible chain, induces a marked increase in specific volume which is expressed as an increase of fluidity (Seelig and Seelig, 1977). The double bond has the greatest effect when it is introduced into a fully saturated chain (Herring *et al*., 1980; Stubbs *et al*., 1981). The degree of unsaturation of phospholipid acyl chain is determined at the level of membrane biogenesis, where fatty acids are selected in the process of phospholipid biosynthesis, and where enzymes manipulate the number of double bonds (Oshino and Sato, 1972) and the length (Dickens and Thompson, 1982) in the available pool of fatty acids (see sections 1.2.2.1 and 1.2.2.2). Exchange processes can also induce a net change in the overall degree of unsaturation (Wirtz and

Zilversmit, 1968; 1969; Jahnig, 1984). The position of the cis double bonds in dienoic fatty acids influences their melting points, the nearer the double bonds to the centre of the chain, the lower the melting point (Christie and Holman, 1967), e.g. linoleic acid (C18:2,ω6) has a melting point of -8°C while C18:2,ω13 fatty acid melts at 34°C. Barton (1975) showed that the phase transition temperatures of a series of phosphatidylcholines synthesized from the complete series of C18:1 fatty acids reveal a similar influence of the position of the double bond.

1.3.3.2 Effects of non-esterified (free) fatty acids (NEFA)

Free fatty acids in membranes may arise from exogenous sources, or from the membrane phospholipids by the action of phospholipases (Stubbs, 1983). The phospholipases A_1 and A_2 are capable of removing the fatty acids attached to the α and β positions of a phospholipid respectively (Van den Bosch, 1980). Due to their amphiphilic nature (containing both hydrophilic and hydrophobic groups); they can partition into different lipid domains of cell membranes (Katz and Messineo, 1983). Klausner et al. (1980) found that cis-unsaturated fatty acids preferentially enter and disrupt fluid areas of the lipid membrane, whereas trans-unsaturated and saturated fatty acids enter the more organized gel regions, where they produce less disruption. Palmitic acid inhibited, whereas oleic acid stimulated sodium dependent calcium uptake by purified cardiac sarcolemmal vesicles (Ashavoid et al., 1985). The physical and biochemical properties of cellular membranes could be influenced by NEFA. In this respect, the interference of NEFA with normal mitochondrial function has been known for several decades (Pressman and Lardy, 1956; Huelsmann et al., 1960; Borst et al., 1962). In a study performed by Piper and coworkers (1983), 10^{-7} to 10^{-6} mol/l of oleate in its unbound form completely abolished mitochondrial oxygen consumption, ATP production and Ca^{2+} uptake. As with esterified fatty acids, the effects of NEFA may be more complex than a mere "fluidizing" of the membranes (Stubbs, 1983).

1.3.3.3 Effects of polar heads of phospholipids

The influence of the phospholipid head groups on the motion of the acyl chain region of the phospholipids has been extensively studied both in model membranes and by supplementation studies with cells in culture. The temperature of the phase transition is sensitive to the type of the head group present and for unsaturated phospholipids, as found in biological membranes, ranges from well below 0°C for phosphatidylcholines to over 30°C for sphingomyelins (Stubbs, 1983).

The detailed studies on the orientation and motional characteristics of the head group region of phospholipids have come from the work of Melchior and Steim (1976). Generally, for all the different head groups, the orientation is almost parallel to the bilayer surface (Seelig and Seelig, 1980), and a restricted internal motion occurs (Akutsu and Seelig, 1981). Comparison of the different head group flexibilities reveals an order: phosphatidylserine < phosphatidylethanolamine < phosphatidylcholine (Browning, 1981). The relationship of these properties to functional characteristics is unclear. However, it has been shown that a conformational change in the head group region accompanies the disordering effect on the fatty acyl chains (Boulanger et al., 1981).

1.3.3.4 Cholesterol

Cholesterol is the major lipid of the plasma membrane of most cells (Stubbs, 1983). It acts as the main lipid rigidifier, above the phase transition, in natural membranes (Shinitzky and Inbar, 1976; Kitajima and Thompson, 1977). Below the lipid phase transition, where a pure lipid bilayer is highly ordered, cholesterol acts in an opposite way - it decreases order and increases fluidity (Hinz and Sturtevant, 1972).

Cholesterol has been assumed to be distributed evenly among the phospholipids, and the molar index of cholesterol/phospholipids (C/PL) could serve as a good qualitative parameter for correlation with the submicroscopic lipid microviscosity (Cooper, 1977).

An important functional effect of cholesterol is to reduce passive permeability to solutes and ions across membranes (Demel et al., 1972). Johannson et al. (1981), however, suggested that the major role of cholesterol in membranes is to act as a buffer to large changes in the physical properties of the membrane by other agents.

1.3.3.5 The protein content

In contrast to the lipid bilayer, proteins are of very low compressibility (Brandt's et al., 1977; Li et al., 1976). The thermal motion of lipids in the vicinity of the proteins is expected to be hindered markedly with a local increase in microviscosity (Shinitzky, 1984). For most membranes the local effect of each individual protein remains limited to the boundary layer of lipids, the "lipid annulus" (Warren *et al.*, 1975), which is highly immobile (Hesketh *et al.*, 1976; Favre *et al.*, 1979), while at more distal lipid domains the effects of proteins converge to a more or less homogenous rigidification (Shinitzky and Inbar, 1976; Cooper, 1977).

1.3.3.6 Membrane fluidity and cellular function

The question of the relationship between membrane fluidity and cell functioning is still under intense investigation. Initial studies were performed on microorganisms (Rottem, 1980; Melchior, 1982), and demonstrated a change in the degree of unsaturation of membrane lipids when growth temperature was varied. In particular, a decrease in temperature resulted in an increase in unsaturation, which suggested a way of maintenance of membrane fluidity at a level optimal for functioning membrane proteins. This process was termed "homeoviscous adaptation" (Sinensky, 1974). Linked to this is the concept that membrane proteins are regulated by membrane fluidity, for which the term "viscotropic regulation" was given (Kimelberg and Papahadjopoulos, 1974; Sandermann, 1978).

Arrhenius plots for many enzyme activities of both micro-organisms and mammalian systems show linear relationships with discontinuities at specific temperatures (Sandermann, 1978). This has been taken as evidence that the particular membrane enzyme involved is regulated by the physical state of the lipid bilayer. In many cases Arrhenius discontinuities have been ascribed to lateral phase separations offering further mechanism for regulation of membrane proteins.

The many attempts aimed at showing that the modulation of cell function brought about by changes in membrane unsaturation is due to changes in membrane fluidity can lead to the hazardous assumption that it is the sole mechanism responsible. The requirement for the lipids to be at least in a "fluid" state has been established for a number of membrane proteins, such as $(Na^+ + K^+)$-ATPase (Kimelberg and Papahadjopoulos, 1972; Abeywardena et al., 1983), Ca^{2+}-ATPase (Hidalgo et al., 1976; Hesketh et al., 1976; Nakamura et al., 1976), Mg^{2+}-ATPase (Buckland et al., 1981) and adenylate cyclase (Salesse et al., 1982a, 1982b). The latter is a particularly interesting membrane enzyme, as it consists of a number of functional subunits capable of independent lateral movement in the lipid bilayer (Lefkowitz et al., 1976). The occupation of a β-adrenoceptor in the membrane outer surface promotes interaction with the catalytic subunit at the cytoplasmic side of the membrane, stimulating the enzyme activity over the basal level (Housley, 1981). The interaction has been termed "collision coupling" and has been found to be enhanced when membrane fluidity is increased (Orly and Schramm, 1975; Hanski et al., 1979).

The question of whether a change in membrane fluidity can exert an effect on the properties of membrane receptors, was addressed by Charnock et al. (1980). They showed a reduced activity of the enzyme that is widely believed to be the receptor for cardiac glycosides, i.e.

myocardial membrane (Na^+ + K^+)-ATPase, in the hearts of winter hibernating ground squirrels. This was associated with increased myocardial membrane fluidity, and a significant increase in linoleic acid content (C18:2,ω6). Ginsberg (1981, 1982), on the other hand, showed that cells enriched in unsaturated fatty acids contained an increased number of insulin receptors and exhibited a decrease in receptor binding affinity. The modulation of the level of unsaturation, however, could have had an effect not mediated through a change in lipid fluidity. At an earlier date Luly and Shinitzky (1979) showed that insulin binding decreased membrane fluidity. This could be a very interesting finding in terms of a better understanding of the probable mechanisms by which hormones and drugs exert an effect at the cellular level.

The mechanism whereby lipids can interact with and influence the properties of certain transporters is not clearly understood. One possibility is that the surrounding lipids affect the conformation of certain carriers, thereby enhancing or reducing the accessibility of their binding sites and if so, then the conformations of only certain transporters are sensitive to lipids, because only in these cases is the structure of protein segment that passes through the lipid bilayer such that it can be affected by lipid modifications (Spector and Yorek, 1985). Also, the lipid microenvironments around various transporters may be different, and then only those transporters located in microenvironments that are changed by a particular type of lipid modification being affected. A lateral phase separation induced by a change in unsaturation (Rintoul *et al.*, 1978; Sanderman, 1978) would create a state of membrane asymmetry, and resulting in differing effects on transporters. Based on the available evidence, however, it seems that all transmembrane carriers may be lipid-sensitive, but only in certain cases are the interactions critical enough to affect transport (Spector and Yorek, 1985).

1.3.4 Eicosanoid Production

Over the last 30 years a considerable research effort has concentrated on the eicosanoids, and great progress has been made in the elucidation of the chemical structure of these compounds, of their physiological roles, and of the ways in which their synthesis is controlled. It is now established that in most tissues the synthesis of eicosanoids is limited by the availability of their common precursor, free arachidonic acid, which must be liberated from esterfied stores in complex lipids (van Dorp *et al.*, 1964; Bergstrom *et al.*, 1964; Vogt *et al.*, 1964; Vonkeman and van Dorp, 1968). The bulk of the arachidonate in mammalian cells is esterified in the fatty acyl chains of glycerophospholipids, almost exclusively in the β-acyl position (Irvine, 1982). This has led to the suggestions that phospholipases are responsible for controlling free arachidonate levels (Lands and Samuelsson, 1968; Van den Bosch, 1980). Phospholipase A_2 is not the only enzyme involved. A phospholipase A_1 followed by lysophospholipase, an active and soluble enzyme, could also effectively liberate arachidonate (Irvine, 1982). Free arachidonic acid is immediately acted upon by the cyclooxygenase enzyme thus converting it to the endoperoxides PGG_2 and PGH_2, which in turn are metabolised to yield prostaglandins, or thromboxanes of the "2" series (Karmazyn and Dhalla, 1982). Leukotrienes, a group of biologically active compounds are also produced from arachidonic acid via 5-lipoxygenase enzyme (Kuo et al., 1984).

The cyclooxygenase enzyme can act on dihomo-γ-linolenic acid (C20:3,ω6) (the precursor of arachidonic acid) to produce prostaglandins of the "1" series (Johnson et al., 1984). Another fatty acid precursor is eicosapentaenoic acid (C20:5,ω3) whose oxidation results in the trienoic prostaglandins and thromboxane A_3 (Moncada and Needleman et al., 1979).

1.4 DIET, LIPIDS AND CORONARY HEART DISEASE

The heart may be affected by a number of pathological processes, but the one extensively studied is myocardial ischemia with its consequences. It may occur as a result of fixed atherosclerotic lesions limiting blood flow above a certain level, or may follow the reduction of myocardial blood flow caused by coronary spasm or platelet aggregates (Hillis and Braunwald, 1978). The clinical sequelae of myocardial ischemia, produced by whatever cause, may be manifested clinically as angina pectoris, electrical instability, depression of myocardial function, and if blood flow is reduced below a certain critical level, irreversible damage to myocardial cells, i.e. myocardial infarction

(Braunwald and Sobel, 1984).

It is important to distinguish between ischemia and hypoxia or anoxia, as they are totally different in their origins and their consequences (Rovetto et al., 1975). In anoxia or hypoxia the oxygen delivery to the myocardium is reduced by removing all or some of the oxygen in the coronary flow. Therefore, while the PO_2 is reduced as in ischemia, coronary flow may be normal or even elevated and substrate delivery and metabolite removal continue to occur (Hearse and Dennis, 1982).

After World War II the upsurge of epidemiologic research on diet, serum lipids, and coronary heart disease (CHD) had significant roots in 18th, 19th and early 20th century medicine (Stamler, 1979). A major breakthrough came from the work of Antischkow in 1908 through 1912, who produced an animal experimental model of human atherosclerosis. This was achieved, by feeding eggs, milk and meat to rabbits to investigate the effects of animal protein on the metabolism and renal function of herbivores (Anitschkow, 1933). It was realized that the rabbits were ingesting animal products rich in cholesterol-fat as well as protein, and the link was made between the dietary lipid, the hypercholesterolemic hyperlipidemia, and the cholesterosis-lipidosis of the atherosclerotic arteries. These important findings highlighted the possibility that atherosclerosis is a lipid metabolic disease, and also the possibility that dietary lipids of animal origin are of major etiological importance. Since then, there have been numerous metabolic studies of the effect of diet modification upon serum cholesterol, triglycerides and various lipoprotein fractions in human subjects confined to metabolic words under close scrutiny (Ahrens, 1957; Keys _et al._, 1957; Hegsted _et al._, 1965). Brown _et al._ (1984) have shown a decline in the death rate from CHD in the U.S., which occurred simultaneously with a reduction in the consumption of cholesterol and saturated fat and an increase in the use of unsaturated vegetable oils. As a result of these and other epidemiological studies, a clear correlation has been established between dietary fats and mortality from CHD due to atherosclerosis (Beare-Rogers, 1984; Wood _et al._, 1984; Arntzenius _et al._, 1985; Kushi _et al._, 1985; Philipson _et al._, 1985; Renaud _et al._, 1985).

Sudden cardiac death did not gain serious attention until the last decade because successful reversal of ventricular fibrillation (VF), its most common cause, was not possible until 1956 when it was established by Zoll _et al._ (1956). The short duration of the event did not allow studies of its causes (and still does not) and it commonly occurs without chest pain or any precise clinical syndrome (Oliver, 1982). The majority of deaths from CHD are sudden and occur within 1 h of last being seen alive and they represent approximately one-third of the total expression of ischemic heart disease (Armstrong _et al._, 1972). It was estimated that there will be about 20-30 deaths per week from sudden cardiac death, mostly younger men, in the majority of populations of one million (Pisa, 1980).

Most cardiologists agree that the aims should be to prevent coronary atherosclerosis and CHD, but these are not the same and should not be equated (Oliver, 1986).

Most basic research presently centres either on the effect that dietary fatty acids might have in regulating platelet aggregation (Gibney, 1982; Ahmed and Holub, 1984; Davenas et al., 1984; Iritani and Narita, 1984; Weiner and Sprecher, 1984) presumably through the production of prostacyclin (PGI_2) or thromboxane A_2 (Vane et al., 1982), or on the association between dietary fatty acids and hypertension (Hoffman and Forster, 1983; Soma et al., 1985). The myocardium itself, although it is the source of pain, dysrhythmias, failure and death, was the focus of considerably less attention.

There is experimental and clinical evidence suggesting that the response of the myocardium to ischemia is determined by the adequacy of substrate availability (Neely and Morgan, 1974) and an optimum ionic equilibrium (Parratt, 1982). Both of these conditions could be affected and altered in different ways. The interest in fatty acids started with the observation that an excess of free fatty acids in the ischemic myocardium leads to profound reduction of oxidative phosphorylation, dysrhythmias and death (Kurien and Oliver, 1970a, 1970b). This was followed by a rising interest in dietary fatty acids and their probable effects on the myocardial function.

1.5 INFLUENCES OF DIETARY FATTY ACIDS ON THE HEART

Diets were shown to influence the heart in ways other than by causing coronary atherosclerosis. Ten Hoor et al. (1973) found that erucic acid (C22:1,ω9) fed to rats for 3 day or 3 week periods, lowered the contractile force of the isolated papillary muscle and the left ventricular stroke work in the heart-lung preparation. In rats rapeseed oil diets produce transient myocardial intracellular lipidosis which peaks at 3 to 7 days. Thereafter this lipid infiltration regresses despite the continued feeding of these diets (Abdellatif and Vles, 1970; Engfeldt and Brunius, 1975). Long-term feeding of diets rich in erucic acid brings about focal myocardial necrosis, chronic inflammatory cell infiltration, and finally fibrosis (Abdellatif and Vles, 1973; Kramer et al., 1973; Charlton et al., 1975; Barer, 1982; Svaar, 1982). Newly introduced rapeseed oil containing low amounts of erucic acid (4% or less) appears to cause focal fibrosis without transient lipidosis (Vogtmann et al., 1975; Beare-Rogers and Nera, 1977; Hulan et al., 1977). More recently, De Wildt and Speijers (1984) showed that after inotropic intervention, only the rapeseed oil fed rats showed less contractile reserve capacity. The absence of this effect in the erucic acid-treated animals was in agreement with the histological studies that showed no fibrotic lesions in these animals. It was concluded that the rapeseed oil itself or some other component but not erucic acid was responsible for loss of contractile reserve capacity. Kramer et al. (1985) further showed that the incidence of lesions was inversely proportional to the levels of saturated fatty acids in the diet, and directly correlated to the C22,ω-3 polyunsaturated fatty acids present in the cardiac membranes.

Information on the role of dietary lipids in determining the composition of myocardial phospholipids accumulated during the 1970's (Beare-Rogers *et al.*, ~~1972a; 1972b~~; Szuhaj and McCarl, 1973; Gudbjarnason and Oskarsdottir, 1975; 1977; Gudbjarnason and Hallgrimson, 1975, 1979; Ruiter *et al.*, 1978). It became evident that while the proportions of fatty acids present in the diet were not reproduced faithfully in the fatty acid distribution in cardiac membrane phospholipids, certain trend~~s were obvious~~. When a diet is rich in a particular fatty acid, that acid appeared in elevated amounts in the membrane phospholipids. Feeding rats for 1-16 weeks diets containing 20% by weight vegetable oils differing widely in their oleic (C18:1,ω9), linoleic (C18:2,ω6) and linolenic (C18:3,ω3) acid content, showed no difference in the concentration of total saturated, C22 polyunsaturated and arachidonic acids in the two major cardiac phospholipids, phosphatidylcholine and phosphatidylethanolamine (Clark, 1980). In the meantime, the incorporation of monosaturated fatty acids was found to depend on their dietary concentration, although the increases were moderate. Substitution of linolenic for linoleic acid-derived C22 polyunsaturated fatty acids was also noted. Further work clearly showed that dietary differences in fatty acid intake altered the fatty acyl tail composition of plasma membrane phospholipids in other tissues as brain, liver and intestinal mucosa (Clandinin *et al.*, 1983). Charnock *et al.* (1983) showed that the fatty acid distribution in cardiac phospholipids was not uniform throughout the heart, but that significant differences in the unsaturated fatty acid proportions existed between atria and ventricles, with the proportions of linoleic (C18:2,ω6) and docosahexaenoic (C22:6,ω3) acids being significantly higher in the latter. The atria, on the other hand, contained higher proportions of oleic (C18:1,ω9), arachidonic (C20:4,ω6) and docosatetraenoic acids (C22:4,ω6). Gibson _et al_. (1984) examined membranes from various organs isolated from rats fed diets in which both the lipid content and composition were varied. They found that despite the large differences in diets there was little effect on the proportion of saturated to unsaturated fatty acids in the phospholipids, the major effect, however, was noticed in the ratio of ω6/ω3 series of unsaturated fatty acids, which increased upon feeding a diet rich in ω6 polyunsaturated fatty acids and decreased when a diet rich in saturated fatty acids was fed. Such an observation was further confirmed by Charnock _et al_. (1985a).

The ability of animals to withstand stress, as induced by acute injections of isoprenaline, was influenced by dietary regimen (Gudbjarnason and Hallgrimson, 1975; 1979). Animals whose diets had been supplemented with 10% cod liver oil, and cardiac membranes contained as a consequence elevated levels of docosahexaenoic acid (C22:6,ω3) suffered 100% mortality, while animals in the control group experienced only 50% mortality. Furthermore, in animals fed a conventional diet, repeated administration of noradrenaline over a period of 15 days led to an increase in the proportion of docosahexaenoic acid in cardiac membrane phospholipids, and the corresponding fall in the proportion of linoleic acid (Gudbjarnason et al., 1978). Similar studies by Crandall et al. (1981, 1982) showed that exercise was necessary to prevent the cardiotoxic action of isoprenaline when sunflower oil (rich in linoleic acid) provided 50% of the calories available in the diet. The cause of death and cardiomyopathy observed in these experiments was thought to be akin to the mechanism proposed by Yates and Dhalla (1975) in which the polyene fatty acids stimulated microsomal oxidation of catecholamines to adrenochrome; and the adrenochrome in its turn stimulated microsomal peroxidation or oxygenation of the polyene fatty acids to various fatty acid derivatives. A similar sequence of events has been postulated to occur in ischemic myocardium and to contribute to the observed pathogenesis (Katz and Messineo, 1981a).

Logan et al. (1977) showed no difference in the incidence of fatal ventricular arrhythmias caused by Ca^{2+} in rats fed for 1 month sunflower seed oil or beef fat (deficient in linoleic acid), while Lepran and co-workers (1981) reported a protective effect of sunflower seed oil, with an increase in the survival rate from 19 to 81% and a reduction in the occurrence of dysrhythmias during the first 20 minutes after coronary ligation. These findings were in direct contrast with the results of Crandall and co-workers (1982) which showed an increased cardiotoxicity of isoprenaline in animals fed diets supplemented with sunflower oil.

Recently, McLennan (1985) investigated the consequences of aging and dietary lipid manipulation on cardiac rhythm under conditions of stress created in anesthetized rats by ligating the left coronary artery. The animals were fed for 6-7 or 18-20 months on either a standard reference diet alone or supplemented (12% w/w) with sunflower seed oil or sheep kidney fat. The number of ventricular extra beats and duration of tachycardia or fibrillation in the 30 min period postligation was increased in sheep kidney fat-fed rats. Infarct size 4 h post-ligation was reduced in sunflower seed oil-fed rats. Dysrhythmias,infarct size, and dietary-induced differences were found to increase with age. These results suggest that age, duration of feeding and the dietary concentration of polyunsaturated fatty acids, are among the factors that might have played a role in the manifestation of the conflicting results previously reported.

Ten Hoor _et al_. (1973) showed that the tension developed by papillary muscles from rats fed for 6 months a diet providing 5% of energy (5 en%) as hardened coconut oil, was significantly lower than that of muscles from animals fed sunflower seed oil in equivalent amounts. Hardened coconut oil is deficient in linoleic acid in contrast to sunflower seed oil. In experiments using isolated working hearts in variations of the Langendorff technique, the same group of investigators showed that even when sufficient linoleic acid was present in both diets, hearts from animals fed 50 en% sunflower oil had greater work capacity without a corresponding increase in oxygen consumption than hearts from animals fed 5 en% sunflower and 45 en% hydrogenated coconut oil. These changes were evident after 5 days of the diet, at which time no appreciable change in the fatty acid profile of the cardiac membrane phospholipids was noted (Vergroeson et al., 1975; De Deckere and Ten Hoor, 1976; Vergroeson, 1977; De Deckere and Ten Hoor, 1979). The authors were reluctant to accept that the differences were attributable to changes in metabolism since the glucose uptake and lactate production were the same in both groups. These results were substantiated in a later report in which lard of unspecified origin was used in place of the hydrogenated coconut oil/sunflower oil mix (De Deckere and Ten Hoor, 1980). Myosin ATPase activities and mitochondrial respiration in the two groups were similar.

Hoffmann et al. (1982) showed that the contractile force of the isolated rat heart was elevated when the linoleic acid content of the diet was raised. After ten weeks of the diet, the spontaneous frequency of the heart preparations used was lower in the group fed the linoleic acid rich diet. After one year of feeding, however, the spontaneous frequencies were similar yet the difference in contractility remained. The release of prostaglandin like substances, and specifically prostacyclin from the heart was increased in the linoleic acid rich diet group.

Charnock et al. (1985b) showed that the positive inotropic responses to Ca^{2+} and the incidence of spontaneous tachyarrhythmias under catecholamine stress in isolated rat papillary muscles, were increased by short-term sheep fat feeding (3-4 months) and with age in control and sheep fat treated groups. Sunflower seed oil prevented these changes. These results demonstrated a marked effect of age upon ventricular myocardial function in the rat which appeared to be accelerated by the consumption of animal (saturated) fat while polyunsaturated vegetable oil provided some degree of protection.

The basis of the observed effects of dietary lipids on the cardiac function is at present unknown, but several mechanisms have been proposed. It has been frequently suggested that changes in the fatty acid composition of membrane phospholipids lead to changes in membrane microviscosity which in turn affect such important functions as transmembrane ion transfer (Katz and Messineo, 1981b; Mead 1984; Stubbs and Smith, 1984). Altered membrane fluidity can also result in changes in membrane-associated enzyme activity or receptor function (Sanderman, 1978; McMurchie _et_ _al_., 1983a, 1983b). Robblee and Clandinin (1984) found that mitochondrial ATPase activity of the rat heart was greatest with 80% saturated fatty acids present in a low fat diet. The activity of the enzyme(s) was reduced by increasing the proportion of polyunsaturated acids, and by increasing the total lipid content of the diet. At the same time, Abeywardena _et_ _al_. (1984) reported no alteration in both the specific activity and the temperature-activity relationship (Arrhenius profile) of the sarcolemmal and sarcoplasmic reticulum ATPases, despite significant changes in membrane composition and physical properties.

An alternative proposition stems from the observation that during dietary manipulations, a constant level is maintained for both the proportion of lipid unsaturation and the value of unsaturation index (Gibson *et al.*, 1984). The major changes were seen in the ratio of (ω-6) family of unsaturated fatty acids (linoleic, 18:2; arachidonic, 20:4) to the (ω-3) family (predominantly docosahexaenoic, 22:6) (Charnock *et al.*, 1983). Rats fed a diet supplemented with sunflower seed oil showed a reduction in ventricular linoleic acid. However, this decrease was accompanied by a significant increase in arachidonic acid. Conversely, rats fed sheep fat supplemented diet manifested a decrease in both linoleic and arachidonic acids, with a major increase (>50%) in cardiac docosahexaenoic acid. A similar increase in (ω-3) fatty acids was found in marmosets fed diets supplemented with mutton fat (Charnock *et al.*, 1985c). Acids of the (ω-3) series are not considered normally to be converted to 3-series prostaglandins in significant amounts nor has any major physiological role been ascribed to them (Willis, 1981). However the (ω-3) acids, and in particular docosahexaenoic acid, are known to be inhibitors of cyclooxygenase (Needleman *et al.*, 1981; Gibney, 1982; Goodnight *et al.*, 1982; Lands, 1982; Corey *et al.*, 1983) and the outcome of the altered myocardial membrane fatty acid composition may be a disturbed balance of eicosanoid production.

1.5.1 The Role of the Eicosanoids

Recent studies have shown that the synthesis of prostaglandins in perfused hearts can be altered by dietary fat manipulation (Ten Hoor *et* *al.*, 1980; Hoffmann *et al.*, 1982) and the effect of linoleic acid rich diet, in reducing mortality following acute coronary artery ligation, was reversed by indomethacin (cyclooxygenase inhibitor) (Lepran *et al.*, 1981). More recently, Charnock *et al.* (1985d) showed that the higher response to Ca^{2+} (change in force of contraction) of papillary muscles from rats fed a linoleic acid poor diet as compared to muscles isolated from animals fed a linoleic acid rich supplement was abolished in the presence of indomethacin, suggesting that differences observed are eicosanoid mediated. Prostaglandins have a wide range of effects on cardiac tissue (Karmazyn *et al.*, 1979; Lefer *et al.*, 1980; Nakaniski *et al.*, 1981; Chiaverelli *et al.*, 1982; Karmazyn and Dhalla, 1983). Depending upon the type of prostaglandin studied, concentration employed and experimental protocol, these compounds can influence the myocardial contractile force and heart rate diversely. In *in vivo* experiments on cats, PGE_1 was found to increase the heart rate and decrease the systemic arterial pressure (Koss *et al.*, 1973). Although PGE_2 had no effects at high doses in the intact cat, in lower doses it exerted a positive inotropic action in the dog (Jones *et al.*, 1974). $PGF_{2\alpha}$ when injected into the coronary artery had no effect on the contractile force of the dog heart (Hollenberger *et al.*, 1968). Similarly, a single bolus injection of $PGF_{2\alpha}$ into the canine sinus node artery produced no chronotropic action (Chiba *et al.*, 1972). High prostacyclin (PGI_2) doses reduced the blood pressure in dogs with a concomitant decrease in heart rate (Chiba and Malik, 1980). Prostacyclin has been reported also to enhance the rate of contraction in isolated heart preparations (Borda et al., 1980).

In addition to chronotropic effects, prostaglandins have also been shown to exert diverse influences on the contractile force of the isolated heart preparations. The effects varied widely from one animal species to another (Vergroesen et al., 1967; Hedqvist and Wennmalm, 1971; Szekeres et al., 1976).

Some prostaglandins are considered to be antidysrhythmic agents and the first report to describe this property was by McQueen and Ungar (1969). Intravenous injection of PGE_1 suppressed dysrhythmic activity due to coronary occlusion in anesthetized dogs (Zijlstra et al., 1972). Coker and Parratt (1983) showed a protective effect of PGI_2 against reperfusion induced dysrhythmia.

Thromboxane A_2 (TXA_2), a product of arachidonic acid metabolism is known to be a potent coronary artery constrictor (Terashita et al., 1978). TXA_2 has been implicated as an important mediator of the electrophysiologic alterations associated with acute myocardial ischemia (Coker, 1982), although this concept is not universally accepted (Kramer et al., 1985).

There has been a growing interest over the past eight years in the role of the trienoic eicosanoids (PGI_3 and TXA_3). It was proposed that while PGI_3 was as effective as PGI_2 in inhibiting platelet aggregation, TXA_3 was only a weak pro-aggregatory agent (Dyerberg, 1978). However, a study by Hornstra et al. (1981) failed to identify any of the 3- series prostaglandins in the platelets and aorta of rats fed fish oil. The mechanism by which trienoic eicosanoids could influence the myocardium is not yet established.

The recent discovery of the leukotrienes (Samuelsson, 1982) has called attention to the effects of the lipoxygenase pathway of arachidonic acid metabolism on the cardiac function. The peptide containing Leukotrienes (LT), i.e. LTC_4, LTD_4 and LTE_4, have been shown to possess a number of effects on the heart including coronary constriction (Letts, 1982), reduced contractility (Bittl et al., 1985) and dysrhythmias (Coker and Parratt, 1984).

1.5.2 Role of Free Fatty Acids

Under conditions of ischemia, free fatty acids, particularly arachidonic acid, are known to accumulate in the myocardium (Prinzen et al., 1984). Free fatty acids have a number of cardiosuppressant actions including $(Na^+ + K^+)$-ATPase inhibition (Miller et al., 1977; Bidard et al., 1984) and suppression of slow action potentials in the hypoxic myocardium (Horada et al., 1984). The effects of different fatty acids from phospholipid pools have not been determined, but a better understanding of their role will provide information on differing susceptibilities of cardiac tissues to inotropic and dysrhythmogenic stimuli.

1.5.3 Role of Free Radicals

The interest in the pathogenesis of acute myocardial infarction has led to an increased awareness of the role played by free oxygen (O_2^-)

and hydroxyl (OH°) radicals in the development of myocardial damage under conditions of acute ischemia or hypoxia (Rao *et al*., 1983a; Burton *et al*; 1984).

The mechanisms by which evolving myocardial ischemia initiates free radical production are not clear. Rao et al. (1983b) suggested the following possibilities: (a) dissociation of intramitochondrial electron transport system with release of ubisemiquinone, flavoproteins and superoxide radicals; (b) accumulation and increased release of intra/extra cellular metabolites like NADH, lactates and catecholamines which react among themselves and with O_2; (c) interaction of the metabolic product hypoxanthine with O_2 in the presence of xanthine oxidase. In a recent study performed on anesthetized dogs, Chambers *et al*. (1985) demonstrated a marked reduction in infarct size following coronary ligation, by using either allopurinol (xanthine oxidase inhibitor) or superoxide dismutase (O_2^- scavenger). This study confirms the importance of the xanthine-hypoxanthine system in generating free radicals, and the role played by the latter in mediating tissue injury following ischemia.

Phospholipase A_2 activity is known to be enhanced by oxygen free radicals (Au *et al*., 1985) and the level of lipid peroxides that accompany free radical generation is higher in lung tissue, if the animals have received a polyunsaturated oil supplemented diet. Since the degree of unsaturation of fatty acids in the membrane is an index of their ability to absorb superoxide radicals and form peroxides, it is possible that differing levels of tetraenoic and hexaenoic acids in membranes render them more susceptible when conditions of O_2^- radical formation exist.

2. RATIONALE FOR PRESENT WORK

Research accomplished to date clearly shows that dietary lipids do exert an effect on the cardiac function. However, the mechanisms by which these dietary constituents can have their influence are poorly understood. The present work is based on the hypothesis that fatty acids from dietary lipids can influence cardiac contractility and susceptibility to dysrhythmia by being inserted into the phospholipids of cell membranes, and exert a regulatory control over excitation-contraction coupling. The object then is to investigate the possible mechanisms whereby this regulatory control is exerted. The experimental steps involved in this study are as follows:

a. Feed rats different lipid supplements, each having a particular fatty acid as a major component.

b. Analyze the fatty acid composition of the phospholipids extracted from both, atria and ventricles.

c. Study and compare the force development of isolated tissues, from the different dietary groups, in response to positive inotropic agents.

d. Determine if the effects are on the contractile elements; this will be done by eliminating the role of membranes using the "chemical skinning" technique.

e. Compare the susceptibility to dysrhythmia in vivo and in vitro.

f. Study the responses of tissues to Bay K8644, a calcium channel agonist, to provide information on the dependence of the cells from the different dietary groups on transmembrane calcium flux.

g. Determine both the uptake of Ca^{2+} by isolated vesicles of sarcoplasmic reticulum (S.R.), as the S.R. plays a pivotal role in regulating contractile tension through calcium release and uptake, and the activity of S.R. ATPase(s).

h. Determine if prostaglandins mediate the dietary effects on contractility and dysrhythmia by pretreatment of tissues with indomethacin prior to and during positive inotropic challenge.

i. Investigate the relationship between free radical production and/or scavenging in the development of inotropy and dysrhythmia in the isolated tissues, using a xanthine oxidase inhibitor and OH° radical scavenger.

j. Assess these hearts histologically for lipidosis and/or necrosis.

3. MATERIALS AND METHODS

3.1 GENERAL

Glass distilled water which was passed through a series of deionizing columns before distillation was used to prepare all solutions. All chemicals used were analytical grade.

3.2 ANIMAL MODEL

The rat was chosen because, besides the convenience of size and cost, its digestion does not include bacterial breakdown of cellulose which, in herbivores, contributes significant amounts of short chain fatty acids to the total absorbed from the intestine. In this respect the rat resembles the human. The rat heart, however, is not typical of mammalian hearts as it develops its greatest contractile force at the lower rates of stimulation; as frequency increases twitch tension falls (Benforado, 1958; Kelly and Hoffman, 1958; Hadju and Leonard, 1959). This "reverse staircase" phenomenon, although not well understood, clearly suggests the involvement of differences in the mechanisms regulating contractile tension in the rat heart, that are not shared by most other mammalian species.

For all experiments 6 to 8-week old male Sprague Dawley rats with an average weight of 158±24 g were used. At the beginning of each experiment the animals were allocated at random to one of four different diets, and fed ad libitum for a period of 9-12 weeks, which was sufficient time for changes to be manifest (Charnock et al., 1983).

3.3 DIET

Four different fat supplements were chosen, each dominated by one of the four classes of fatty acids. Oleic (C18:1,ω9) in rapeseed oil (CAN) (West Canola - Canbra Food Ltd., Lethbridge - Alta.), linolenic (C18:3,ω3) in linseed oil (LIN) (Alberta Linseed Oil Co., Medicine Hat - Alta.), linoleic (C18:2,ω6) in sunflower seed oil (SFO) (Safflo - CSP Food Ltd., Saskatoon - Sask.), and saturated fatty acids in sheep perirenal fat (SKF) (Lambco, Innisfail - Alta.).

Diets were prepared according to the method of Charnock et al. (1983), where standard laboratory pellets (Ralston Purina Canada Inc.) were soaked overnight in either of the three oils, then drained and stored at 4°C. The sheep perirenal fat was processed by heating in an autoclave for 15 min which was followed by homogenization in a domestic blender, and steaming for another 15 min. The fat was then filtered into ice-cold water, collected and stored at 4°C. For preparing the diet, the fat was melted and maintained at 60°C while pellets were added and allowed to soak for 30 min before being allowed to drain and cool. Retention of oil by the CAN and SFO diets was less than LIN and SKF as the viscosities of these oils were lower. The final fat content and energy supplied by fat (en% see table 1) of each diet were within 10% of the mean of the group. The diet was then stored at 4°C.

The final fat contents of these diets (% w/w) were:

CAN	LIN	SFO	SKF
20.76	25.29	19.56	23.43

Analysis of the diets has confirmed that they were well above the minimum requirements for protein, vitamins, minerals and energy set by the National Research Council of the U.S.A., Subcommittee on Laboratory Animal Nutrition (1978). Due to the fact that protein and carbohydrate/fat ratios were altered from normal commercial feed, a group of rats receiving no lipid supplement would not have served as a control. The experiments were controlled by comparison among the groups.

3.4 ISOLATED TISSUES

At the end of the feeding period animals were weighed, then sacrificed by decapitation under light ether anesthesia. The hearts were rapidly removed and washed free of blood in ice-cold Bretag's solution (Bretag, 1969), containing 1.53 mM Ca^{2+}, bubbled with 95% O_2 - 5% CO_2 mixture. The atria were dissected free from the ventricles. The right ventricle and the inter-ventricular septum were cut and the left ventricle laid flat to expose the papillary muscles. Two left ventricular papillary muscles were isolated from most hearts. After separation from the left atrium, the right atrium and sections from the left ventricle were kept under liquid nitrogen prior to lipid analysis. The rest of the left ventricle was preserved in 10% buffered formalin for histological examination.

The isolated tissues were mounted on lucite holders in contact with punctate platinum electrodes, which were then immersed in tissue baths containing Bretag's solution at 37°C, gassed with 95% O_2 - 5% CO_2 mixture.

Tension development was measured using U.F.I. isometric force displacement dynamometers (Searle Bioscience), and displayed on either a Grass model 5 or model 7 polygraph (Grass Instruments, Quincy, Mass.). Tissues were stimulated at 1 Hz by square-wave pulses of 5 ms duration at supramaximal voltage from a Grass 5D9 stimulator. After 30 min equilibration, the length-tension relationship was determined using a vertically mounted precision micrometer to apply 0.25 mm increments in tissue length. When maximal twitch tension was reached the length was adjusted so that the twitch tension equalled 70% of maximum. After equilibration for a further 30 min one of the following experiments was performed on one set of tissues. At the end of an experiment the relaxed length of each tissue was measured, and the weight was determined after blotting off excess fluid.

3.4.1 Calcium and Isoprenaline

A concentration-effect curve was obtained to the cumulative addition of Ca^{2+} after 20 min equilibration in "Ca^{2+}-free" Bretag's solution. No Ca^{2+} chelating agent was used. The bathing solution was then returned to normal with fresh Bretag's solution containing 1.53 mM Ca^{2+}. After 30 min equilibration, isoprenaline was added in a cumulative fashion to determine the concentration-effect relationship. Drugs were added using a 10 µl (Hamilton) or 1 ml syringe at 5 min intervals.

In the course of the experiments it was noted that some left atria and papillary muscles developed spontaneous extra twitches (dysrhythmia) between the electrically driven beats. The threshold concentrations of inotropic agent for the induction of dysrhythmia (spontaneous consecutive contractions for 15 s or more) were determined for both Ca^{2+} and isoprenaline.

3.4.2 Indomethacin

The previous experiment was performed in the presence of 0.01 mM indomethacin which was added to the tissue bath 30 min prior to addition of the inotropic agent. Indomethacin was prepared in a stock solution of 1 M in Tris - buffer (pH 8.5).

3.4.3 Allopurinol

The influence of 1 μM allopurinol (a xanthine oxidase inhibitor) (Chambers et al., 1985) on the dysrhythmogenic effects of isoprenaline in isolated tissues, was studied by adding allopurinol to the tissue bath 30 min prior to commencing the cumulative addition of isoprenaline.

3.4.4 Mannitol

This drug was used as an OH° radical scavenger at 20 mM concentration (Dorfman and Adams, 1973), and it was left to act for 30 min prior to the cumulative addition of isoprenaline.

3.4.5 Bay K8644

Concentration - effect curves were obtained to the cumulative addition of Bay K8644. It has been shown that the amplitude of the positive inotropic effect evoked by Bay K8644 was increased in isolated rat heart tissues by lowering the external Ca^{2+} concentration (Finet *et al.*, 1985). For this reason, experiments were conducted in Bretag's solution in which Ca^{2+} was reduced to 0.75 mM. Tissues were allowed 30 min to equilibrate at this lower Ca^{2+} concentration before drug addition. The drug was dissolved in polyethylene glycol (400). All results were compared to solvent control experiments. Precautions were taken to prevent exposure of the drug solutions to light by wrapping all containers with aluminium foil.

3.5 CHEMICAL SKINNING

Treatment of rat cardiac tissues with 10 mM EGTA renders the sarcolemma highly permeable to small ions and molecules without removing its restriction of the diffusion of larger molecules or inactivating all of its enzymatic functions (McClellan and Winegrad, 1978). These workers developed such a technique to overcome the limitation inherent in the study of intact cells. The contractile properties of the protein could not be directly assayed *in situ* as the excitation-contraction coupling mechanism could not be bypassed. As a result it was not possible to correlate the inotropic responses of the tissue with any alteration in the sensitivity of contractile proteins to calcium.

The heart was removed and transferred to oxygenated ice-cold

Bretag's solution. The left atrium was dissected free, and silk threads were secured through the ends. Silk threads were also placed around the ends of left-ventricular papillary muscles. The isolated tissues were then pinned to a sylgard slab, taking care to keep the length approximately that in the unstretched heart. The tissues were exposed to the disruption solution (containing 10 mM EGTA) at 0°C for 48 h. The EGTA-treated tissues were then transferred to the standard organ baths with volumes of 3.5 ml, and attached to force transducers in the same manner as in previous experiments. Using the micrometer screw assembly the length of each tissue was adjusted to resting length against a millimetre-scale attached to the lower end of the lucite holder. Tissues were allowed to equilibrate at pCa 9.00 for about 15 min or until a steady state tension developed, at a temperature of 37°C. Changes in the medium were made by upward displacement utilizing freshly prepared "contracture" solution with varying free Ca^{2+} concentration calculated according to Fabiato and Fabiato (1979). Chart-paper was set at a speed of 0.25 mm/sec, and tissues were given sufficient time for tension development before the next exposure to a higher concentration of free Ca^{2+}. At the end of each experiment tissues were blotted dry and weighed.

3.6 CORONARY LIGATION

Anesthesia was induced by intraperitoneal injection of pentobarbitone sodium, 6 mg/100g (Somnotol, M.T.C. Pharmaceuticals).

Small additional amounts were administered intravenously as required. The rat was then laid supine on the operating table with the four limbs taped down. Body temperature was monitored using a rectal thermometer, and was maintained at approximately 38°C.

The skin on the inner aspect of one thigh was cut, and the femoral vein exposed and separated from the femoral artery using blunt dissection along the axis of the blood vessels. A double thread was passed beneath the vein and the distal portion tied. Using a fine pair of scissors a small cut was made in the vein wall and the bevelled end of a fine nylon catheter was passed inside the vein and tied in place. The cannula was attached to a needle, which in turn was attached to a 3-way stopcock. A 10 ml syringe containing heparinised saline (100 units/ml) and a 1 ml syringe filled with pentobarbitone were also attached to the stopcock.

The skin of the neck was cut transversely and was followed by a longitudinal cut to the top of the sternum. The trachea was exposed with a longitudinal blunt dissection of the sternohyoid muscle and intubated. The common carotid artery was then identified on one side, and after a careful dissection from the vagus a double thread was placed, and the rostral portion was tied close to the head. After placing a small artery clamp as caudal as possible a small nick was made in the vessel wall close to the rostral ligature, and a cannula was passed down the lumen. The caudal ligature was tied loosely. The clamp was then removed and the cannula pushed down the artery a further centimeter, and it was secured in place by tying a second knot. The cannula was fitted to a 3-way stopcock to which a Statham-P23 blood pressure transducer (Statham, Hatorey, Puerto Rico) and a 10 ml syringe (with heparinised saline) were attached. A small quantity of the heparinised saline was pushed down the artery before switching the pressure transducer into the circuit. Prior to each experiment the calibration of the pressure transducer was checked against a mercury manometer. The blood pressure and E.K.G. (lead I) were recorded on a Washington 400 MD2C oscillograph (George Washington Ltd., Sheerness, Kent, England) using two couplers (Narco Biosystems, Houston, Texas). Heart rate was monitored using Narco Biosystems Digital Display DD-350.

Blood pressure and heart rhythm were allowed to stabilize for about 15 min. A left thoracotomy was performed by cutting through the 4th, 5th and 6th ribs. The animal was started on mechanical ventilation with room air (stroke volume, 4 ml; 48 strokes/min) using a rodent respirator (Harvard Apparatus, Millis, Mass.). Once the blood pressure and E.K.G. were stable, the heart was exteriorized, using a narrow stainless steel loop, and a 6/0 silk suture (Ethicon, Somerville, N.J.) was placed under the main left coronary artery near its origin from the aorta as described by Selye et al. (1960). The heart was then replaced in the chest and allowed to stabilize for 15 min at the end of which the coronary ligature was tightened. At the end of a 30 min observation period, the animal was sacrificed by infusing 1 ml of pentobarbitone.

The chest was then opened and after clamping the thoracic aorta a solution of 2% malachite green was perfused into the carotid artery to delineate the ischemic area as a check on the success of the ligation.

3.7 SARCOPLASMIC RETICULUM (S.R.) STUDY

3.7.1 S.R. Isolation

The method of Entman et al. (1973) was used. Animals were sacrificed at the end of the feeding period by decapitation under light ether anesthesia. The hearts were removed and the atria dissected free from the ventricles in an ice-cold bicarbonate-azide buffer (isolation medium, pH 7.0). Atria from seven animals were pooled together to yield enough S.R. fraction, while ventricles were dealt with separately. Tissues were chopped into small pieces with a pre-chilled razor blade, and then homogenized in 25 ml of isolation medium using two 10 s bursts at setting 6 on the Polytron. The homogenate was centrifuged at 4000 x g for 20 min (temp 2°C). The supernatant was collected and recentrifuged at 8700 x g for 20 min (2°C). The pellet was discarded and the supernatant centrifuged at 100,000 x g for 35 min (2°C). The high speed pellet was harvested and resuspended in 20 mM Tris-maleate and 0.6 M potassium chloride (pH 6.8) in order to remove contractile protein contaminants and recentrifuged at 100,000 x g and 2°C for 35 min. The final pellet was resuspended in 20 mM Tris-maleate (pH 7.1) and used within 1 h of preparation.

3.7.2 S.R. Ca^{2+} Uptake and ATPase Activity

Oxalate supported Ca^{2+} uptake by the S.R. was measured at 37°C using a calcium electrode (Madeira, 1975). The equipment consisted of a Radiometer F2112 Ca^{2+} electrode (Radiometer, Copenhagen), a pH electrode (Ingold Electrodes Inc., Andover, Mass.)., a Radiometer-M64 pH meter, a Corning pH/ion meter 135 (Corning Glass Works, Medfield, Mass.), an antilog converter, a DC power supply (Hewlett Packard 6H2A), digital thermometer (Thermalent Model TH-6, Sensortek Inc., Clifton, N.J.), a Linear chart-recorder (Linear Instruments, Irvine, Calif.). The reactions were conducted in a thermostatically controlled vessel with a magnetic stirring rod. The reaction medium comprised 10 mM KCl, 0.5 mM $MgCl_2$, 20 mM Tris-maleate, 5 mM oxalate, 5 mM sodium azide (NaN_3), 4 mM Mg-ATP. Each reaction volume (2.138 ml) contained initially 80 nmole Ca^{2+}, present as chloride. Three steps of 200 nmole OH^- (2 µl of 0.1 M KOH) were used as a pH calibrtation. For calibration of the calcium electrode, a further 20 nmole Ca^{2+} (2 µl 10 mM $CaCl_2$ solution) was added and the resulting potential change noted. Sufficient time was allowed for the Ca^{2+}-sensitive electrode to equilibrate (10-15 min). The final volume was 2.146 ml at pH 7.10. A 50 µl aliquot of the S.R. fraction was added, and the uptake of Ca^{2+} from free solution over time was recorded as voltage change.

The ATPases activity was determined by continuous monitoring of pH changes after addition of the S.R. fraction. Hydrolysis of an ATP molecule yields one proton as follows:

$$ATP^{-} + H_2O \xrightarrow{\text{ATPase}} ADP^{-} + \text{Phosphate}^{-} + H^{+} + 7.3 \text{ Kcal/mole}$$

Measurements were performed in the absence (total ATPases) and presence (Ca^{2+}-independent ATPases) of 0.2 mM EGTA, and the difference between both provided information on the Ca^{2+}-dependent ATPases. At the end of each experiment the total amount of protein in the fraction studied was estimated.

In these experiments the unusual step of including a reference group of animals was taken. The reference animals were of similar age to the experimental ones, but were fed a diet of rat chow without lipid supplement. This group was included only as a calibration of the methodology, and to allow comparison with the results obtained from the rat in laboratories where this was an established technique. The present results were compatible with those obtained by Belke and Wang (personal communication).

3.7.3 Protein Assay

The method of Bradford (1976) was used with slight modification. A concentrated dye reagent containing Coomassie Brilliant Blue G, phosphoric acid and methanol, obtained from Bio-Rad (Rich. - California), was used after a four-fold dilution with reaction medium. This solution was then filtered through Whatman No. 1 paper and stored for not more than 2 weeks at room temperature.

Standard protein solutions were freshly prepared from a stock solution of 1 mg per 1 ml bovine serum albumin (98%, Sigma) to provide concentrations of 10, 25, 50, 75 and 100 µg protein/ml.

The assay was performed by adding 1 ml of either the sample or standard to 4.1 ml of diluted dye reagent. Mixing was done by gentle inversion of the tubes. After a period of five min to 1 h, optical density at 595 nm (OD_{595}) was measured versus reagent blank on a Beckman 2400 Spectrophotometer (Beckman Instruments Inc., Fullerton - Calif.). Reagent blank was prepared by adding 1 ml reaction medium to 4.1 ml diluted dye reagent.

The optical densities at 595 nm were plotted against concentrations of standards, and the unknowns were read from the standard curve.

3.8 FATTY ACID ANALYSIS

3.8.1 Preparation of Fatty Acid Esters from the Diet

Lipids were extracted by soxhlet extraction to constant weight using chloroform as solvent. Chloroform solutions were evaporated under reduced pressure in a stream of nitrogen.

Fatty acids were esterified by the method of Christie (1982), whereby the lipid sample (0.5 ml) was dissolved in benzene (0.5 ml) and 2% sulfuric acid in methanol (5 ml) was added. The mixture was refluxed for 2 h, then water (5 ml) containing sodium chloride (5%) was added and the required esters were extracted with hexane (2 x 5 ml) using disposable Pasteur pipettes to separate the layers. The hexane layer was washed in water (4 ml) containing potassium bicarbonate (2%). The solution was filtered and the solvent removed under reduced pressure in a stream of nitrogen.

To rid the sample of attached impurities a silicic acid column was employed. Silicic acid was dried at 160°C overnight (approx. 16 h). The column was prepared by pouring silicic acid in hexane (10 g silicic acid per 100 mg sample) into a Pasteur pipette. The sample was applied in hexane and the column washed with 3 column volumes of hexane. The esters were eluted with 3% petroleum ether (BP 35-60) in hexane (v/v). The solvent extract was dried and analysed.

3.8.2 Preparation of Fatty Acid Esters from the Tissues

The method described by Charnock et al. (1983) was followed. Approximately 50 mg wet weight of right atrial or left ventricular tissue was dispersed into 20 ml of ice-cold buffer (20 mM Tris methylamine, 1 mM EDTA, 250 mM sucrose, pH 7.6) by two 10 sec bursts with a polytron tissue disintegrator (Kinematical GmbH, Switzerland) at setting 5. The tissue brei were centrifuged at 78,000 g for 30 min at 2°C. The supernatant was then discarded and the tissue pellet resuspended in ice-cold water and centrifuged twice to remove sucrose prior to extraction of the lipids.

Lipids were extracted by the method of Folch et al. (1957) using 20 vol of chloroform:methanol (2:1) which contained 0.01% (w/v) butylated hydroxy-toluene (BHT) as antioxidant. The extract was dried under a stream of nitrogen. The total phospholipids were separated using a silicic acid column (Pearce and Kakulos, 1980). Silicic acid was dried, as previously described, and a slurry of 4 g was poured into the column (23 mm in length and 15 mm in diameter) and washed with 15 ml chloroform. The dried solvent extract was dissolved in 1 ml chloroform and applied to the column. Neutral lipids were eluted first using 30 ml chloroform containing 0.05% BHT. The phospholipids were then collected by applying 55 ml chloroform:methanol (1:9) to the column. The solvent was evaporated under negative pressure in a stream of nitrogen. The total phospholipid fraction was then dissolved in 0.5 ml of benzene, and methyl esters were prepared according to Charnock *et al*. (1983) by heating in 2 ml methanol containing 14% (w/v) borontrifluoride in sealed vials for 30 min at 60°C. After cooling, 3 ml of water was added and the fatty acid methyl esters were extracted twice with 5 ml of petroleum ether (BP 35-60). The solvent extract was dried and analyzed.

3.8.3 Gas Liquid Chromatography

The dried extract was analyzed using a Hewlett-Packard gas chromatograph (model 5730A) fitted with a flame ionization detector and column of 10% DEGS (Supelco Inc., Bellafonte, PA) together with a Hewlett Packard integrator (model 3390A) for calculation of the peak area. Extracts were redissolved in 0.5 ml of dry hexane and injected as 1 µl aliquots. The carrier gas was nitrogen (flow rate of 28 ml/min) and the column temperature was maintained constant at 200°C. Fatty acids were identified from their relative retention times compared to those obtained for authentic methyl fatty acid standards.

3.9 HISTOLOGICAL TECHNIQUES

Left ventricles were dissected free and fixed in 10% neutralized formol-saline.

3.9.1 Hematoxylin and Eosin

The method described by Kiernan (1981) was used.

3.9.1.1 Solutions required

a. Mayer's hemalum

The following were dissolved, in the order given, in 750 ml of distilled water:

Aluminum potassium sulfate	50.0 g
Hematoxylin (C.I. 75290)	1.0 g
Sodium iodate	0.1 g
Citric acid (monohydrate)	1.0 g
Chloral hydrate	50.0 g

The final volume was made up with distilled water to 1000 ml. This solution can be stored for several months.

b. Eosin

Eosin (C.I. 45380)	2.5 g
Distilled water	500.0 ml

This solution keeps indefinitely and may be used repeatedly.

3.9.1.2 Staining procedure

Paraffin sections were de-waxed and hydrated, then stained for 2-5 min in Mayer's hemalum. This was followed by a washing step in running tap water for 2-3 min or until the sections turned blue. The wet slides were examined under the microscope to check for the nuclear staining. Slides were then immersed in eosin for 30s with agitation, and washed in running tap water for another 30s. Dehydration followed in 70%, 95%, and two changes of absolute ethanol (with agitation, about 30s in each change). The sections were cleared in xylene and mounted with permount (Fisher Scientific Co. Inc.).

3.9.2 Fat Studies

Oil red O and Sudan black B were used to stain and show neutral fat deposits (Luna, 1968).

3.9.2.1 Solutions required

a. Oil red O Solution

oil red O	0.5 g
Propylene glycol 100%	100.0 ml

A small amount of propylene glycol was added to the oil red O and heated gently to 95°C. The solution was then filtered through coarse filter paper while still warm. It was allowed to stand overnight at room temperature, and was filtered again through Seitz filter with the aid of vacuum.

b. Sudan Black B Solution

0.7 g Sudan black B was dissolved in 100 ml of propylene glycol by heating to 100°C and stirring thoroughly for a few minutes. It was filtered hot through Whatman No. 2 paper to remove excess dye. After cooling to room temperature it was filtered again through a Seitz filter with the aid of vacuum.

c. Buffered Neutral Formalin Solution (BNF)

37-40% formalin	100.0 ml
Distilled water	900.0 ml
Sodium phosphate monobasic	4.0 g
Sodium phosphate dibasic	6.5 g

3.9.2.2 Staining procedure

Cardiac tissues were fixed in buffered neutral formalin solution and then frozen. Frozen sections were cut 8 μm in thickness and picked onto gelatin subbed slides. They were air dried for several hours and fixed to the slides with BNF for 5 min, then rinsed with distilled water. Equilibration was done with 85% propylene glycol for 5 min. Staining was carried out overnight in either 0.5% Oil red O or 0.7% Sudan black B solutions. This was followed by a differentiation step in 85% propylene glycol for 5s. The slides were then rinsed well in distilled water and mounted with glycerine jelly.

3.10 CHEMICALS

Allopurinol, ascorbic acid (disodium salt), ATP (disodium salt), ATP (magnesium salt), creatine phosphate (disodium salt), creatine phosphokinase (type I), EDTA (disodium salt), EGTA, heparin (disodium salt), indomethacin, isoprenaline (hydrochloride), malachite green (oxalate salt), mannitol, oxalic acid (potassium salt) and Tris-maleate were all purchased from the Sigma Chemical Co., Missouri, U.S.A.

Aluminium potassium sulfate, buffered formalin (10%), calcium chloride, chloral hydrate, chloroform (HPLC grade), citric acid, diethyl ether, dimethyl sulfoxide, eosin, glucose, hexane (HPLC grade), magnesium chloride, magnesium sulfate, methanol (HPLC grade), paraffin (Paraplast Plus), permount, potassium chloride, potassium hydroxide, propylene glycol, sodium azide, sodium bicarbonate, sodium chloride, sodium hydroxide, sodium iodate, sodium phosphate (monobasic) and sucrose were all purchased from Fisher Scientific Co. Inc., New Jersey, U.S.A. British Drug Houses Ltd. (Poole, England) provided the following: boron trifluoride methanol complex (14%BF_3), butylated hydroxy-toluene, sodium gluconate and Tris (hydroxymethyl) methylamine. The Bay K8644 was kindly provided by Miles Pharmaceuticals (West Haven, CT.). Hematoxylin was purchased from Fluka AG, Buchs SG, Switzerland. Ethanol was purchased from C.I.L. (Montreal, Que.). Imidazole was the product of Eastman Kodak Co. Ltd. (Rochester, N.Y.). Oil red O was obtained form Polysciences Inc. (Warrington, PA.). Petroleum ether (BP

35-60) was purchased from J.T. Baker (Philipsburg, N.J.). Silicic acid was obtained from Bio Rad (Richmond, CA.). Sodium pentobarbitone(Somnotol) was obtained from M.T.C. Pharmaceuticals (Hamilton, Ont.) and Sudan black B was purchased from Harleco (Philadelphia, PA.).

All other chemicals were purchased from either BDH Ltd., or Fisher Scientific Co. Inc.

3.11 SOLUTIONS

3.11.1 Bretag's Solution

The final composition of the synthetic interstitial fluid (S.I.F.) was as follows:

Component	Molar concentration (mM)		by weight (g/l)
NaCl	107.70		6.30
KCl	3.48		0.26
$CaCl_2$	1.53	($CaCl_2.2H_2O$ 10% solution)	1.70 ml
$MgSO_4$	0.69	($MgSO_4.7H_2O$)	0.17
$NaHCO_3$	26.20		2.20
NaH_2PO_4	1.67	($NaH_2PO_4.H_2O$)	0.26
Na gluconate	9.64		2.10
glucose	5.55		1.00
sucrose	7.60		2.60

The solid components were dissolved in close to the final volume of distilled, deionized water. The solution was then equilibrated with carbogen (5% carbon dioxide in oxygen). Finally, the calcium chloride (as a 10% solution was added and the solution topped up to its total volume. In use, and with continuous bubbling with carbogen the S.I.F. had a pH of 7.2 at 37°C.

3.11.2 Disruption Solution

Component	Molar concentration (mM)		by weight (g/1)
K-propionate	140	(1 M solution)	140.00 ml
Mg-acetate	2		0.43
EGTA	10		3.80
Na_2 ATP	5		2.76

pH was adjusted to 7.2 with 5 mM imidazole. 1 M K-propionate solution was prepared by mixing equal volumes of 2 M propionic acid and 2 M potassium hydroxide.

3.11.3 Contracture Solution

Two solutions at pCa 9.00 and 4.50 were prepared and mixed in order to obtain other pCa values. These two solutions were prepared from the following stock solutions that could be stored frozen at -20°C for two weeks in polyethylene bottles.

a. Solution I (pCa 9.00)

Stock solution	Volume
5 mM $K_2CaEGTA$	1.02 ml

100 mM K_2EGTA	14.96 ml
0.5 M $MgCl_2$	2.54 ml
1 M KCl	8.72 ml
50 mM Na_2ATP	9.90 ml

1 M KOH for adjusting pH to 7.10

The solution was brought up to its final volume of 150 ml with water.

5 mM $K_2CaEGTA$ was prepared with 0.50 g $CaCO_3$, 5 ml 1M EGTA and 10 ml 1 M KOH made to a final volume of 1 litre with water.

100 mM K_2EGTA was prepared with 100 ml 1M EGTA and 200 ml 1 M KOH made to a final volume of 1 litre with water.

b. Solution II (pCa 4.50)

Stock Solution	Volume
100 mM $K_2CaEGTA$	19.92 ml
5 mM K_2EGTA	1.64 ml
0.5 M $MgCl_2$	2.82 ml
1 M KCl	11.88 ml
50 mM Na_2ATP	13.24 ml

1 M KOH for adjusting pH to 7.10

The solution was brought up to its final volume of 200 ml with water.

100 mM $K_2CaEGTA$ was prepared with 10.01 g $CaCO_3$, 100 ml 1 M EGTA and 200 ml 1 M KOH made to a final volume of 1 litre with water.

5 mM K_2EGTA was prepared with 5 ml 1 M EGTA and 10 ml 1 M KOH made

to a final volume of 1 litre with water.

The volumes of solutin I and solution II given below were mixed to obtain 50 ml aliquots of solutions at the other pCa values.

	Solution I	Solution II
pCa 9.00	50.00 ml	00.00 ml
pCa 7.00	39.90 ml	10.10 ml
pCa 6.00	11.25 ml	38.75 ml
pCa 5.50	4.06 ml	45.94 ml
pCa 5.00	1.15 ml	48.85 ml
pCa 4.50	0.00 ml	50.00 ml

Creatine phosphate and creatine phosphokinase were added in solid form to the experimental solutions just before they were used.

3.11.4 Isolation Medium

Component	Molar concentration (mM)	by weight (g/l)
$NaHCO_3$	10	0.840
NaN_3	5	0.325

pH was adjusted to 7.0 with 1 M KOH.

This solution was freshly prepared before each experiment, and the bottle was kept all the time in ice.

3.11.5 Salt Washing solution (for removal of contractile proteins)

Component	Molar concentration (mM)	by weight (g/l)
Tris-maleate	20	4.744
KCl	600	44.736

3.11.6 Reaction Mixture

Component	Molar concentration (mM)	by weight (g/l)
KCl	100.0	7.456
$MgCl_2$	0.5	0.407
Tris maleate	20.0	4.744
K-oxalate	5.0	0.831
NaN_3	5.0	0.325
Sucrose	100.0	34.230

The same mixture was prepared with the presence of 0.2 mM EGTA (76.08 mg/l) as a calcium chelator.

3.12 ANALYSIS OF RESULTS

When possible the results are given as the mean ± standard error of the mean.

The possible effects of dietary lipids on weight, blood pressure, heart rate, twitch tension response to agonists, uptake of calcium by S.R. and ATPases activity and fatty acid composition of membranes were compared using an analysis of variance with Duncan's multiple range test modified for unequal sample sizes (Wallpole, 1981; Dowdy and Wearden, 1983). Unpaired t-test was used to compare the effects of one dietary treatment on twitch tension response to agonists, in the presence or absence of indomethacin. A paired t-test was used to analyze the maximal increases in tissues mean twitch tension to Bay K8644.

Differences in the percentage of tissues becoming dysrhythmic under challenge with inotropic agents were analyzed using χ^2 or Fisher's exact probability test where appropriate (Siegel, 1956). A probability value of 5% or less ($P<0.05$) was selected as indicating a significant difference.

4. RESULTS

4.1 FATTY ACID CONTENT OF THE SUPPLEMENTED DIETS

The fatty acid composition of the diets is shown in Table 1. Sheep fat diet was relatively rich in saturated fatty acids while oil supplemented diets were rich in unsaturated fatty acids. The rapeseed oil diet was a rich source of oleic acid (C18:1,ω9) (60.4%) while sunflower oil diet contained a high proportion of linoleic acid (C18:2,ω6) (70%). Linolenic acid (C18:3,ω3), on the other hand, was a major constituent of linseed oil diet (70.6%). In addition to its relatively high content of palmitic acid (C16:0) (19.7%) and stearic acid (C18:0) (24.2%), sheep fat diet contained a substantial amount of oleic acid (45.3%) and ranked second after rapeseed oil diet as a source of this ω-9 fatty acid.

4.2 BODY WEIGHTS

Following a feeding period of 9-12 weeks, there was no significant difference in the weight gained by animals in any of the four dietary groups (Table 2). n = 9 (in each group).

4.3 BLOOD PRESSURE AND HEART RATE

The mean arterial blood pressure was recorded in anesthetized rats via intracarotid cannula, and heart rate was determined from E.C.G. tracing. There was no significant difference in either of these parameters among the dietary groups following a feeding period of 9-12 weeks (Table 3). n = 10 (in each group).

Table 1. Energy density and fatty acid content of the lipids present in supplemented diets.

	*Energy density of diet	*Energy derived from lipid	Fatty Acids							%Sat.	Unsat. Index**
			Saturated			Unsaturated					
						ω-9		ω-6	ω-3		
	kJ/g	en%	16:0	18:0	20:0	18:1	20:1	18:2	18:3		
Rapeseed oil	17.7	28.1	3.3	0.7	-	60.4	0.9	23.5	9.6	4.0	137.1
Sheep fat	18.7	34.2	19.7	24.2	0.2	45.3	-	5.8	0.7	44.1	59.0
Linseed oil	18.1	33.5	1.8	0.9	-	10.2	-	16.0	70.6	2.7	254.0
Sunflower oil	18.1	28.8	6.9	4.5	-	17.9	-	70.0	-	11.4	157.9

Fatty acid values are percent total fatty acids.

* Combustion calorimetry performed by Mrs. L. Kwan-Yeung.

** Calculated from the proportion of each unsaturated acid multiplied by the number of double bonds it contained, all summated.

Table 2. Mean body weight of rats ± S.E.M. before and after feeding fat augmented diets for 9-12 weeks. No significant difference was observed among the groups (P>0.05; Duncan's multiple range test). n=9 (in each group).

Dietary Group	Body Weight (g) Initial	 At Sacrifice
Rapeseed oil	149 ± 6.73	501 ± 24.13
Sheep fat	164 ± 6.84	495 ± 14.56
Linseed oil	159 ± 9.78	487 ± 14.43
Sunflower seed oil	158 ± 8.7	496 ± 14.48

Table 3. Mean arterial pressure and heart rate of rats in the four dietary groups following a feeding period of 9-12 weeks. Measurements were done using an E.K.G. and an intracarotid cannula. No significant difference was observed among the groups (P>0.05; Duncan's multiple range test). Values are mean ± S.E.M. (n=10, in each group).

Dietary Group	Mean Arterial Blood Pressure (mmHg)	Heart Rate (beats/min)
Rapeseed oil	133 ± 9.33	356 ± 7.85
Sheep fat	136 ± 7.56	377 ± 13.79
Linseed oil	132 ± 7.81	353 ± 13.21
Sunflower seed oil	136 ± 7.68	361 ± 11.25

4.4 FATTY ACID COMPOSITION OF ATRIAL AND VENTRICULAR PHOSPHOLIPIDS

Cardiac phospholipids arise predominantly from the membranes of cardiac cells (Witting _et al_., 1961; Kramer, 1980).

The total phospholipid fatty acid composition of the right atria and left ventricles from rats in the different dietary groups is shown in Tables 4 & 5. Although no statistical comparison was done between atria and ventricles from a single dietary group, a trend similar to that observed by Charnock _et al_. (1983) was noticed. The proportions of linoleic acid (C18:2,ω6) and docosahexaenoic acid (C22:6,ω3) were higher in the ventricles, while the proportions of oleic (C18:1,ω9) and docosatetraenoic acid (C22:4,ω6) were higher in the atria irrespective of the diet. The proportion of arachidonic acid (20:4,ω6), however, was not appreciably different in any tissue, atria or ventricles, in any dietary group examined. For both tissues the ratio of saturated: unsaturated fatty acids did not differ markedly among the groups despite the different levels of unsaturation in the diets (Gibson _et al_., 1984). However, the proportions of certain fatty acids changed significantly with diet. In the atria the proportion of palmitic acid (C16:0) was (24%) higher in the CAN-dietary group in comparison to the LIN-dietary group. The dimethylacetal derivative of stearic acid (C18:0 DMA) showed a significantly higher level in atria from SKF-group in comparison to the other three groups, although this accounted for only 2.2% of the total. The ventricles, on the other hand, did not manifest significant differences in the proportions of individual saturated fatty

Table 4. Fatty acid composition of total phospholipids of right atria from four groups of rats fed fat augmented diets for 9-12 weeks. Significance was tested using Duncan's multiple range test ($P<0.05$). Values are percentages of total fatty acids (mean ± S.E.M.).

CAN = rapeseed oil; SKF = sheep fat; LIN = linseed oil; SFO = sunflower seed oil. N.S. = not significant.

DMA = dimethylacetal

Fatty Acid	CAN(n=7)	SKF(n=6)	LIN(n=9)	SFO(n=6)	Significance (P<0.05)
16:0 DMA	0.89±0.20	1.24±0.17	1.34±0.28	1.31±0.26	N.S.
16:0	10.75±0.19	9.96±0.89	8.19±1.04	9.53±0.68	CAN>LIN
18:0 DMA	0.75±0.19	2.20±0.31	1.35±0.21	1.42±0.27	SKF>CAN,LIN& SFO;SFO>CAN
18:0	24.64±0.31	26.36±0.78	25.74±0.61	26.49±1.13	N.S.
20:0	0.31±0.06	0.17±0.04	0.22±0.03	0.19±0.03	N.S.
16:1,ω7	0.39±0.06	0.54±0.05	0.25±0.04	0.25±0.06	SKF>LIN&SFO
18:1,ω9	13.88±0.28	10.47±0.22	10.44±0.29	8.03±0.27	CAN>LIN&SKF> SFO
20:1,ω9	0.19±0.02	-	-	-	-
18:2,ω6	12.08±0.42	8.32±0.67	12.60±0.48	12.59±0.55	CAN,LIN&SFO> SKF
20:3,ω6	0.76±0.06	0.81±0.07	0.82±0.03	0.99±0.07	N.S.
20:4,ω6	20.98±0.62	22.53±0.57	20.76±0.65	23.65±0.41	SFO>CAN&LIN; SKF>LIN
22:4,ω6	1.92±0.46	1.64±0.08	0.96±0.07	4.74±0.41	SFO>CAN&SKF> LIN
22:5,ω6	-	0.35±0.04	—	1.37±0.21	-
18:3,ω3	1.25±0.20	0.37±0.03	1.86±0.11	0.40±0.04	LIN>CAN>SFO& SKF
22:5,ω3	2.92±0.37	2.70±0.24	5.11±0.29	1.04±0.10	LIN>CAN&SKF> SFO
22:6,ω3	6.49±0.24	10.14±0.47	7.13±0.28	6.16±0.24	SKF>CAN,LIN& SFO
ΣSat.	37.01±0.86	39.84±1.35	36.26±0.72	38.70±0.48	
Σω7,ω9	14.28±0.24	11.28±0.39	10.72±0.34	8.20±0.32	
Σω6	36.25±0.51	33.20±1.53	35.16±0.98	43.12±0.67	
Σω3	10.17±0.40	13.21±0.66	12.98±1.09	7.53±0.27	
ω6/ω3	3.56	2.51	2.71	5.73	
Unsaturation index*	189.80	203.96	199.14	200.27	

*Calculated from the mean proportion of each unsaturated acid multiplied by the number of double bonds it contained, all summated.

Table 5. Fatty acid composition of total phospholipids of left ventricles from four groups of rats fed fat augmented diets for 9-12 weeks. Significance was tested using Duncan's multiple range test ($P>0.05$). Values are percentages of total fatty acid content (mean ± S.E.M.).

CAN = rapeseed oil; SKF = sheep fat; LIN = linseed oil; SFO = sunflower seed oil. N.S. = not significant.

DMA = dimethylacetal

Fatty Acid	CAN(n=7)	SKF(n=8)	LIN(n=10)	SFO(n=6)	Significance (P<0.05)
16:0 DMA	0.77±0.11	0.94±0.2	1.35±0.19	1.02±0.21	N.S.
16:0	8.54±0.29	8.76±0.8	8.00±0.84	8.39±0.37	N.S.
18:0 DMA	0.98±0.11	1.54±0.26	1.22±0.13	1.26±0.24	N.S.
18:0	23.10±0.25	23.65±0.27	25.37±0.64	24.80±0.34	N.S.
20:0	0.15±0.03	-	0.09±0.02	-	-
16:1,ω7	0.29±0.05	0.52±0.03	0.28±0.03	0.25±0.05	SKF>CAN,LIN& SFO
18:1,ω9	10.36±0.49	7.43±0.66	7.18±0.65	4.54±0.22	CAN>LIN&SKF> SFO
20:1,ω9	-	0.12±0.01	-	-	-
18:2,ω6	18.83±0.74	16.28±0.54	20.39±0.72	21.16±0.81	LIN&SFO>SKF
20:3,ω6	0.51±0.02	0.58±0.03	0.59±0.05	0.48±0.04	N.S.
20:4,ω6	19.03±0.47	20.78±0.62	19.33±1.17	21.23±0.57	N.S.
22:4,ω6	0.42±0.06	0.4 ±0.04	0.34±0.08	1.14±0.11	SFO>CAN,LIN& SKF
22:5,ω6	0.13±0.01	0.21±0.03	-	1.23±0.10	SFO>CAN&SKF
18:3,ω3	0.81±0.06	0.29±0.03	2.15±0.04	0.22±0.02	LIN>CAN>SFO& SKF
22:5,ω3	2.17±0.24	1.95±0.04	3.34±0.32	1.02±0.06	LIN>CAN&SKF> SFO
22:6,ω3	12.45±0.60	15.15±0.56	10.55±0.49	10.15±1.66	SKF>CAN,LIN& SFO
ΣSat.	33.46±0.56	34.79±1.09	35.71±0.93	35.48±0.68	
Σω7,ω9	10.68±0.48	7.95±0.64	6.90±0.45	4.82±0.28	
Σω6	38.82±0.79	38.11±0.61	39.42±1.00	45.17±0.54	
Σω3	15.43±0.48	17.38±0.59	15.92±0.60	13.06±0.35	
ω6/ω3	2.52	2.19	2.48	3.46	
Unsaturation index*	206.47	175.26	213.78	210.84	

*Calculated from the mean proportion of each unsaturated acid multiplied by the number of double bonds it contained, all summated.

acids under dietary influence.

The major changes were observed with unsaturated fatty acids. In the atria and ventricles palmitoleic acid (C16:1,ω7) was significantly higher in the SKF-group. Such a difference, however, is unlikely to have significance in terms of membrane function because of the low content of this fatty acid (0.39%). Animals given the CAN diet had significantly higher atrial and ventricular oleic acid (C18:1,ω9) than in the LIN- and SKF-groups while in the SFO, it was present in the lowest proportion of all. Linoleic acid content of atria and ventricles did not differ significantly among rats fed vegetable oil supplemented diets despite the differing levels of this fatty acid in these diets, but it was significantly lower in tissues of the SKF-group. However, arachidonic acid (C20:4,ω6) content of atria did not differ statistically between SFO- and SKF-groups, which were at the same time significantly higher than CAN- and LIN-dietary groups. Ventricles, on the other hand, did not show appreciable changes in levels of arachidonic acid with dietary manipulation. Animals in the SFO-group showed higher atrial and ventricular levels of the further products of ω-6 metabolism (C22:4,ω6) and (C22:5,ω6) in comparison to the other groups.

Linolenic acid (C18:3,ω3) and 22:5,ω3 fatty acid were both significantly higher in atria and ventricles of animals in the LIN-group in comparison to the other groups. Eicosapentaenoic acid (C20:5,ω3) could not be detected in any tissue, atria or ventricles, in any dietary group examined. Docosahexaenoic acid (C22:6,ω3), on the other hand, was significantly higher in atria and ventricles of animals in the SKF-group when compared to the other dietary groups.

Tissues, atria and ventricles, from LIN- and SKF-groups did not show marked differences in the total content of each of the three unsaturated groups of fatty acids (Σω3; Σω6; Σω7,ω9) despite the wide variation in dietary availability. However, the highest content of ω-3 fatty acids was observed in these groups in comparison to CAN- and SFO-groups. Tissues from the CAN-group, on the other hand, reflected the higher dietary content of ω-9 as an increased Σω7,ω9 in comparison to the other three dietary groups. Atria and ventricles from SFO-group showed the highest content of ω-6 fatty acids, and the least ω-3 and ω-9. It appears that there exists a reciprocal relation between tissue content of ω-6 fatty acids, and both ω-3 and ω-9 fatty acids. The ω6/ω3 was markedly higher in atria from the SFO-group, and to a lesser extent in ventricles.

4.5 CONTRACTILE RESPONSES OF ISOLATED HEART TISSUES FROM DIFFERENT DIETARY GROUPS TO INOTROPIC STIMULI

4.5.1 Length-Tension Relation

The relation between tissue length and developing tension is represented as least squares linear regression for atria (Fig. 1) and ventricles (Fig. 2) in each dietary group. When the mean lines derived in these figures were directly compared, there was no difference in the

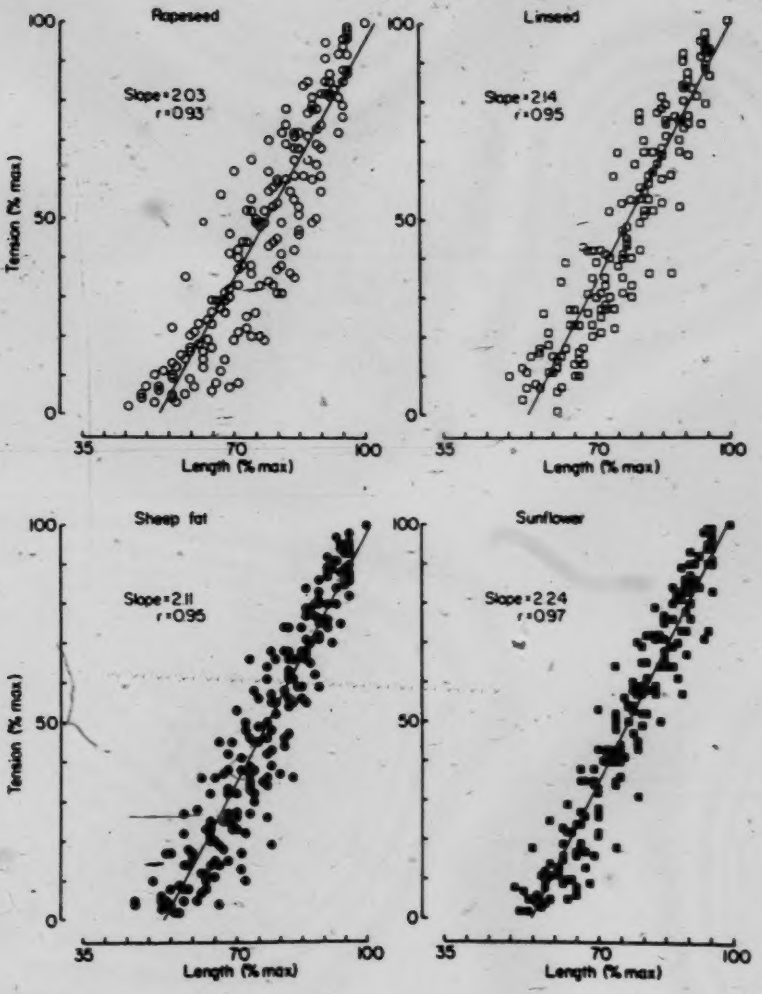


Fig. 1. Normalized length-tension relation of left atria from four groups of rats fed fat augmented diets for 9-12 weeks. The symbols in each panel represent data from 19 preparations. The lines are the least squares linear fit for the points. The slopes and the correlation coefficients are as indicated.

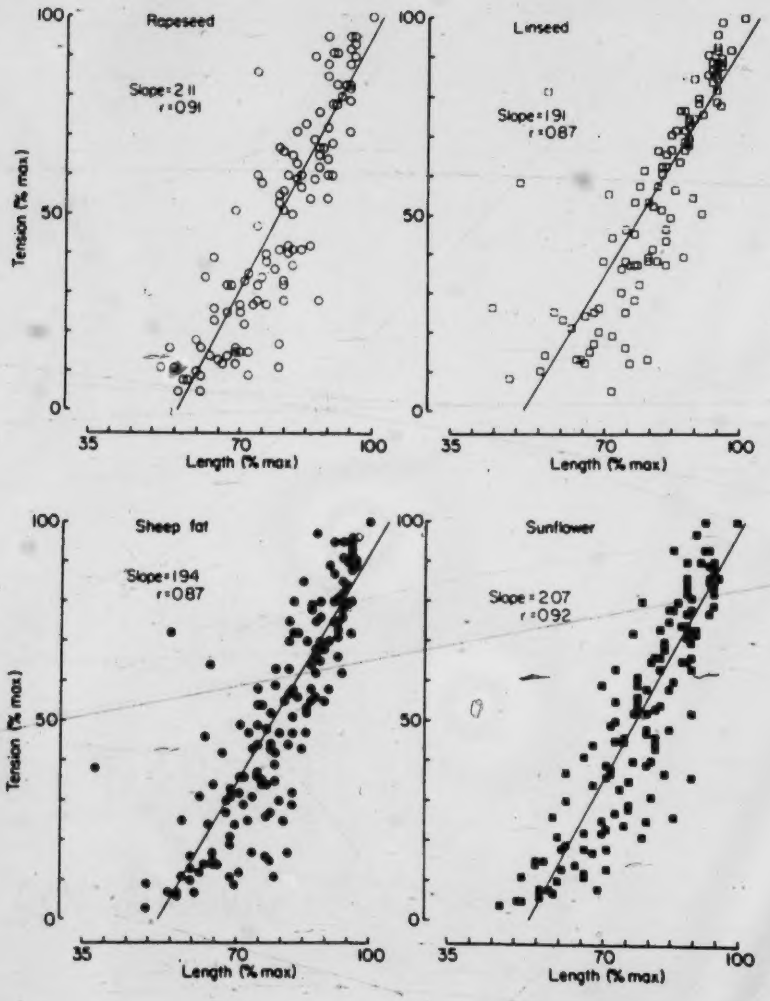


Fig. 2. Normalized length-tension relation of papillary muscles from four groups of rats fed fat augmented diets for 9-12 weeks. The symbols in each panel represent data from 19-23 preparations. The lines are the least squares linear fit for the points. The slopes and the correlation coefficients are as indicated.

slopes with the lines being almost superimposible (Fig. 3).

4.5.2 Effect of Ca^{2+} on Force of Contraction in Isolated Heart Tissues

Following the equilibration of tissues for 20 min in Ca^{2+}-free Bretag's solution prior to the cumulative addition of Ca^{2+}, there was no contracture or loss of muscle function which are characteristics of the calcium paradox. This corresponds with the results of others who reintroduced calcium in a graded fashion spread over a period of time (Charnock et al., 1985b).

Left atria for SKF-fed rats exhibited significantly greater increases in twitch tension in response to Ca^{2+} than did left atria from the other three groups ($P<0.05$) (Fig. 4). The maximum increase in tension was 221 μN/mg tissue for SKF; 111 μN/mg tissue for CAN; 105 μN/mg tissue for LIN and 86 μN/mg tissue for SFO.

Papillary muscles from the same animals displayed different responses to Ca^{2+}, with the tension developed by tissues from CAN-fed animals showing significantly lower values than tissues from the other three groups ($P<0.05$) (Fig. 4). The maximum increase in tension was 169 μN/mg for SKF; 148 μN/mg for SFO; 140 μN/mg for LIN and 62 μN/mg for CAN.

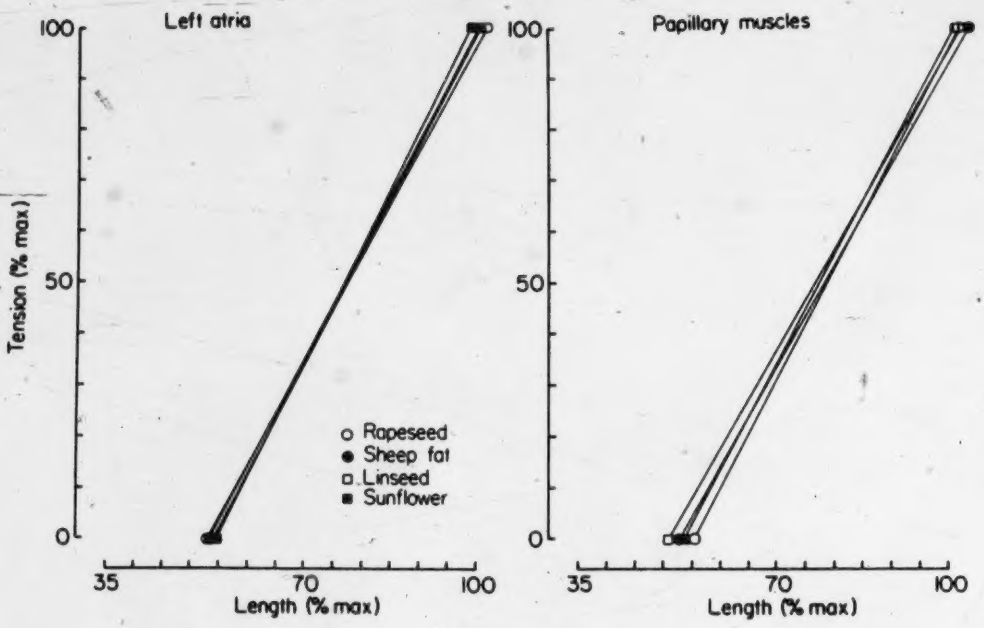


Fig. 3. Normalized length-tension relation of left atria and papillary muscles from the four dietary groups of rats represented as the least squares linear fit.

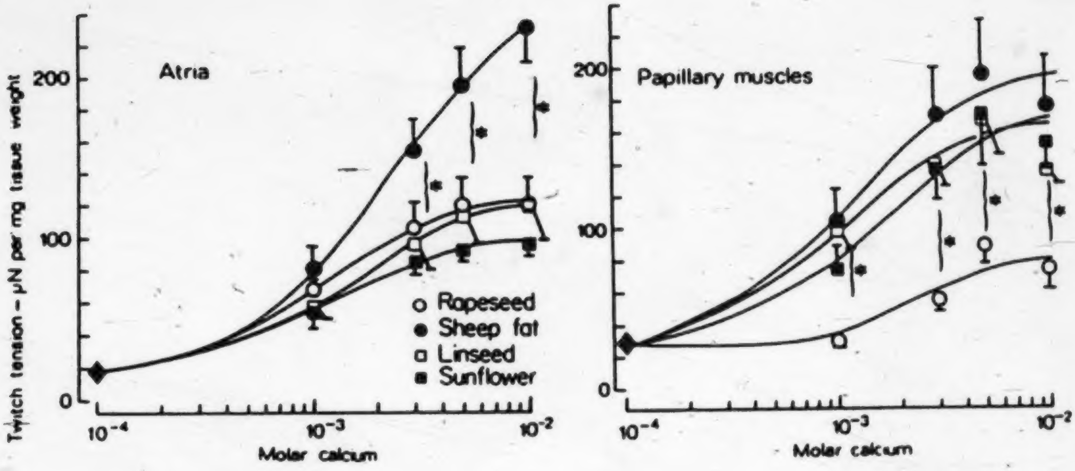


Fig. 4. Concentration vs twitch tension curves for calcium on left atria and papillary muscles from four groups of rats fed fat augmented diets for 9-12 weeks. Asterisk (*) indicates a significant difference ($P<0.05$; Duncan's multiple range test). Results are mean ± S.E.M. (n= 9-13, left atria: n = 6-14, papillary muscles).

4.5.3 Effect of Ca^{2+} on Tension Development in "Chemically Skinned" Isolated Heart Tissues

The purpose of this study was to compare the sensitivity of the contractile proteins to calcium in heart tissues isolated from animals in the different dietary groups. According to McClellan and Winegrad (1978) the cardiac fibre that has been made hyperpermeable by EGTA maintains the following experimental properties: (a) the surface membrane is a minimal diffusion barrier to small ions and molecules; (b) the cells retain a high level of activity of soluble enzymes; (c) the effect of intracellular Ca^{2+} sinks, especially the sarcoplasmic reticulum, on the sarcoplasmic concentration of Ca^{2+} is small because 10 mM EGTA in the bathing solution is an effective Ca^{2+} buffer; (d) some of the sarcolemmal enzymes are still functional in spite of the altered state of the membrane; and (e) the preparation is sufficiently stable to allow meaningful comparisons of performance within a single experiment.

When EGTA treated left atria and papillary muscles were challenged with increasing concentrations of Ca^{2+}, the previously observed differences in contractile force were abolished (Fig. 5). It should be noted, however, that the maximum tension developed by skinned left atria was only about 20% of that seen in the weakest group of electrically stimulated intact tissues. Skinned papillary muscles from SKF-and LIN groups developed a maximum tension which was about 50% of that seen in the electrically stimulated intact tissues, while skinned tissues from the CAN-group displayed a maximum tension similar to that seen in intact

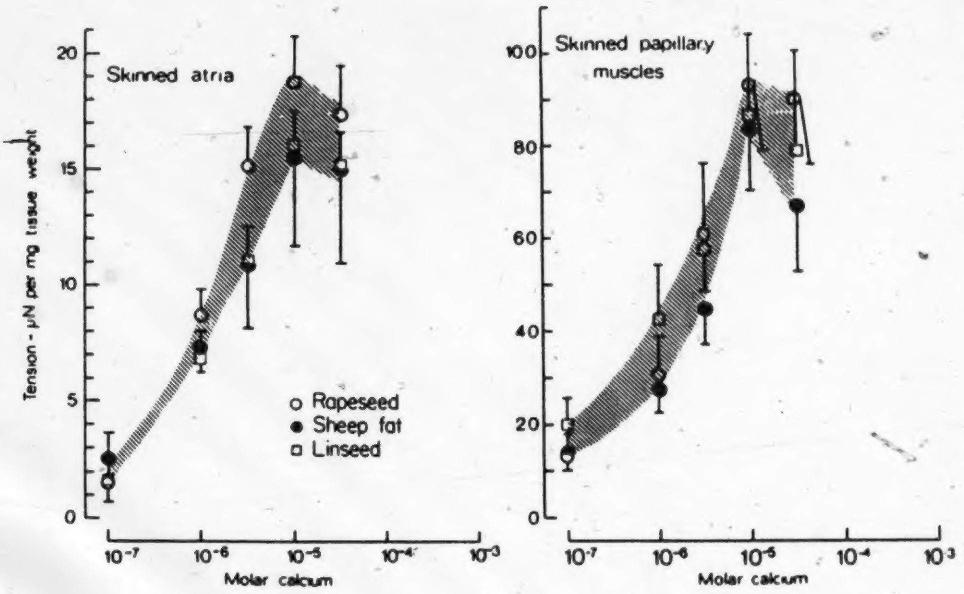


Fig. 5. Concentration vs tension curves for calcium on chemically skinned left atria and papillary muscles from three groups of rats fed fat augmented diet for 9-12 weeks. No significant difference was observed between the groups ($P>0.05$; Duncan's multiple range test). Results are mean ± S.E.M. (n = 9-12, left atria; n = 8-11, papillary muscles).

papillary muscles.

4.5.4 Effect of Indomethacin on Twitch Tension Development to Ca^{2+} in Isolated Heart Tissues

Indomethacin effectively inhibits the cyclooxygenase enzyme system (Vane, 1971). In the presence of 0.01 mM indomethacin, the significant differences in the responses of left atria and papillary muscles from the four dietary groups to Ca^{2+}, seen in Fig. 4, were abolished (Figs. 6 and 11). However, the presence of indomethacin did result, at the highest concentration of Ca^{2+} (10 mM), in a reduction of twitch tension of left atria which was statistically significant ($P<0.05$) (Figs. 7,8,9 and 10). Papillary muscles from the CAN-group in particular, behaved differently as mean tension developed in the presence of indomethacin was higher than in its absence, although as noted above such a difference was not statistically significant (Fig. 12). Papillary muscles from the remaining dietary groups, on the other hand, manifested reduced responses to calcium in the presence of indomethacin which was statistically significant ($P<0.05$) for SKF- and LIN-groups (Figs. 13 and 14), evident at the highest concentration of Ca^{2+} (10 mM). Although mean tension values in the SFO-group were lower in the presence of indomethacin, this was not statistically significant (Fig. 15). However, the small number of remaining tissues that had not developed spontaneous dysrhythmia ($n=3$) at the two highest concentrations of Ca^{2+} prejudiced statistical evaluation of the differences seen.

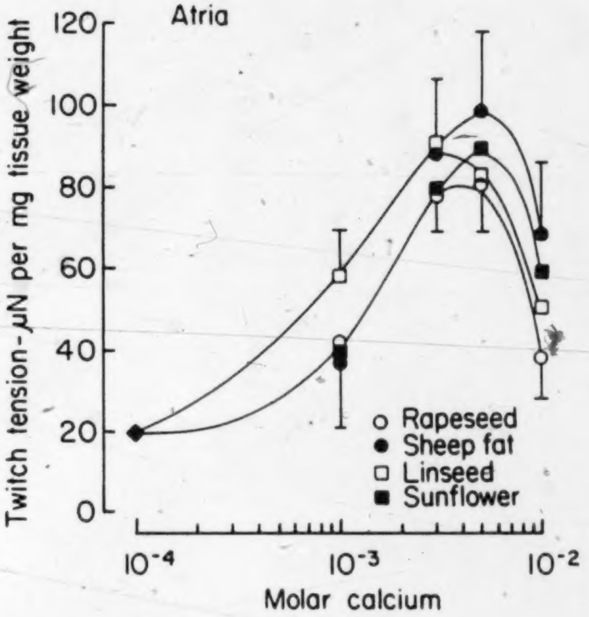


Fig. 6. Concentration-effect curves of calcium in the presence of 0.01 mM indomethacin on left atria from four groups of rats fed fat augmented diets for 9-12 weeks. Indomethacin was added 30 min prior to the addition of calcium. No significant difference was observed between the groups ($P>0.05$; Duncan's multiple range test). Results are mean ± S.E.M. ($n = 7-12$).

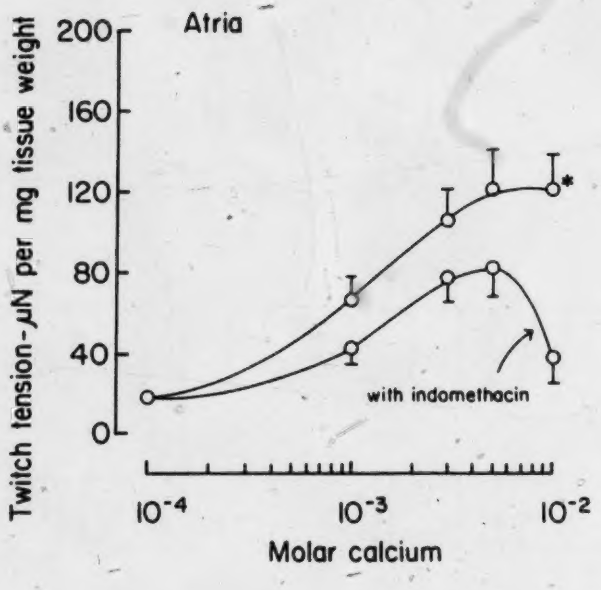


Fig. 7. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on left atria from rats fed Rapeseed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 10-12).

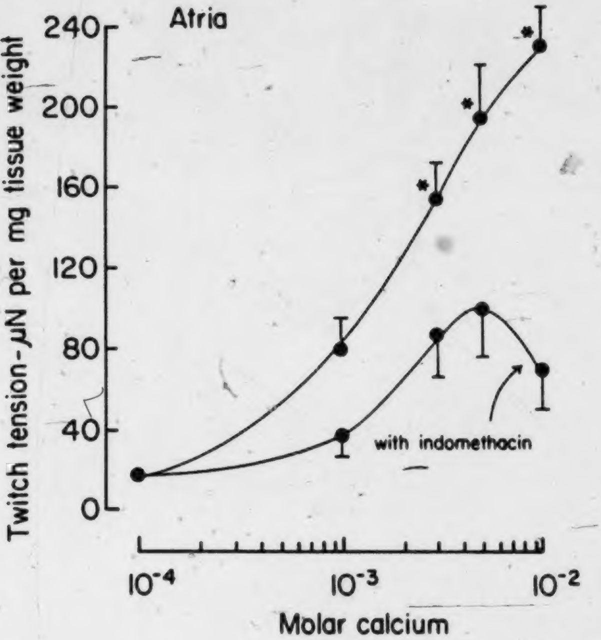


Fig. 8. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on left atria from rats fed sheep fat supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 7-11).

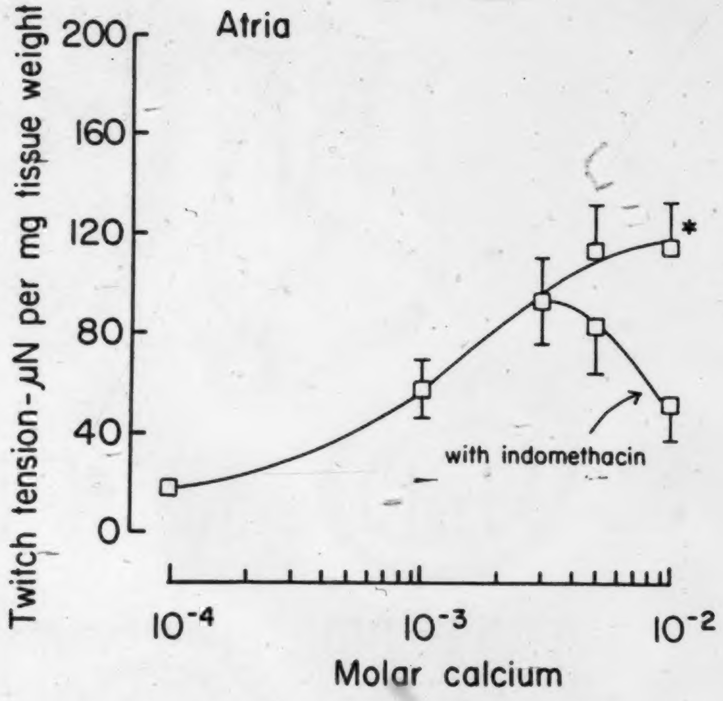


Fig. 9. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on left atria from rats fed linseed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Student's unpaired t-test). Results are mean ± S.E.M. ($n = 12$).

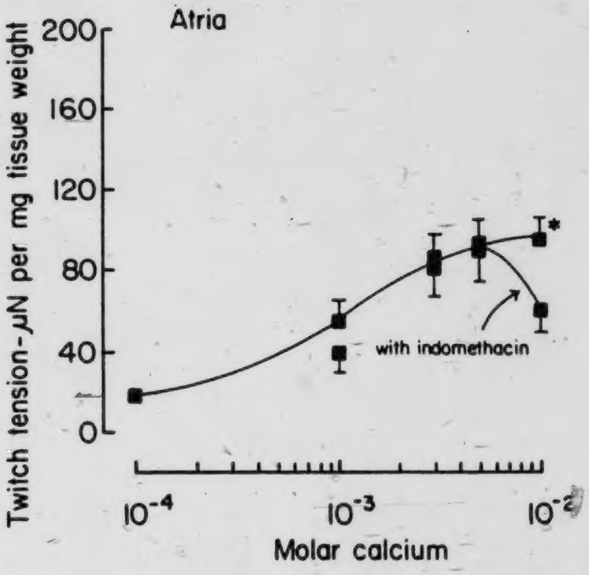


Fig. 10. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on left atria from rats fed sunflower seed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 12-13).

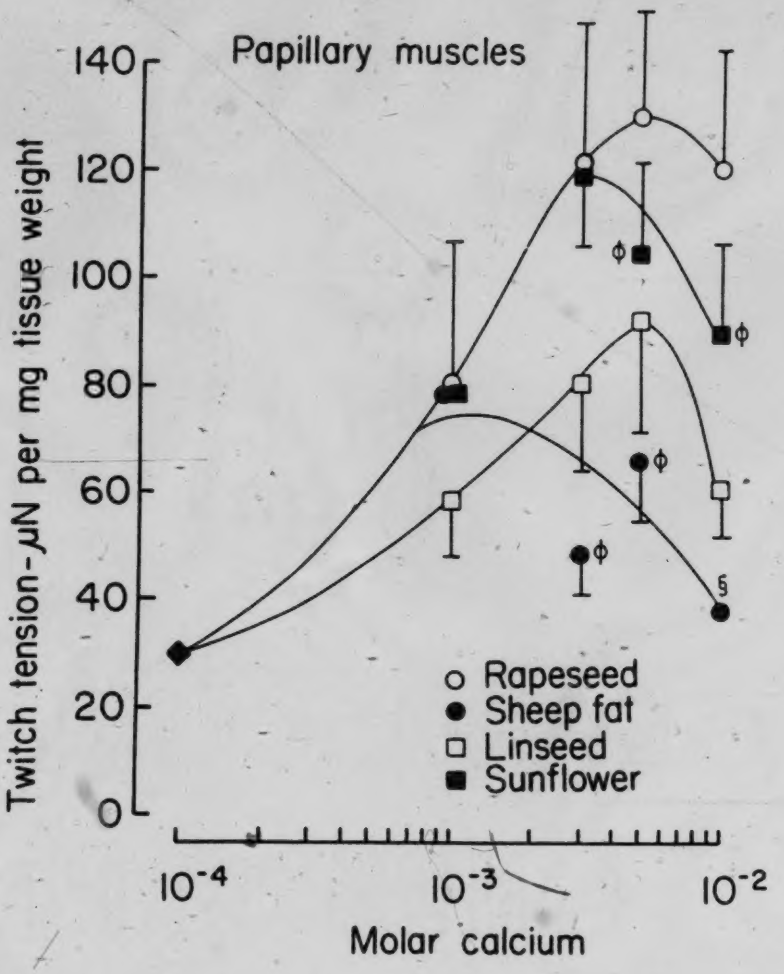


Fig. 11. Concentration-effect curves of calcium in the presence of 0.01 mM indomethacin on papillary muscles from four groups of rats fed fat augmented diets for 9-12 weeks. Indomethacin was added 30 min prior to the addition of calcium. No significant difference was observed between the groups (P>0.05; Duncan's multiple range test). Results are mean ± S.E.M. (n = 6-14; §n = 1; ϕn = 3).

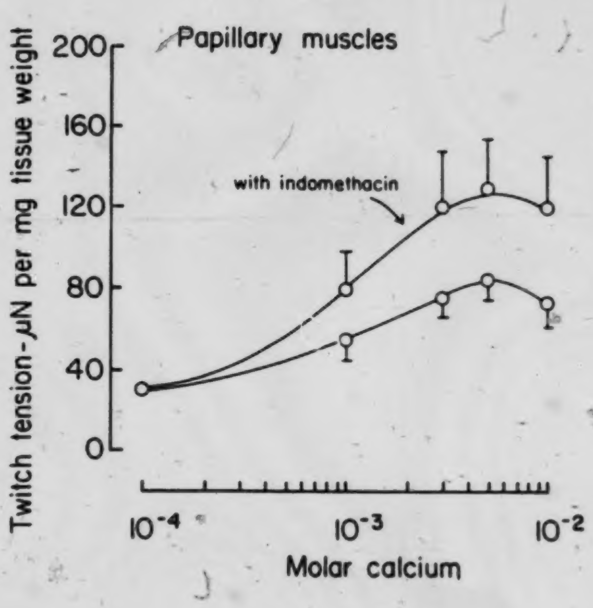


Fig. 12. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on papillary muscles from rats fed rapeseed oil supplemented diet for 9-12 weeks. No significant difference was observed between the two curves ($P>0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 9-13).

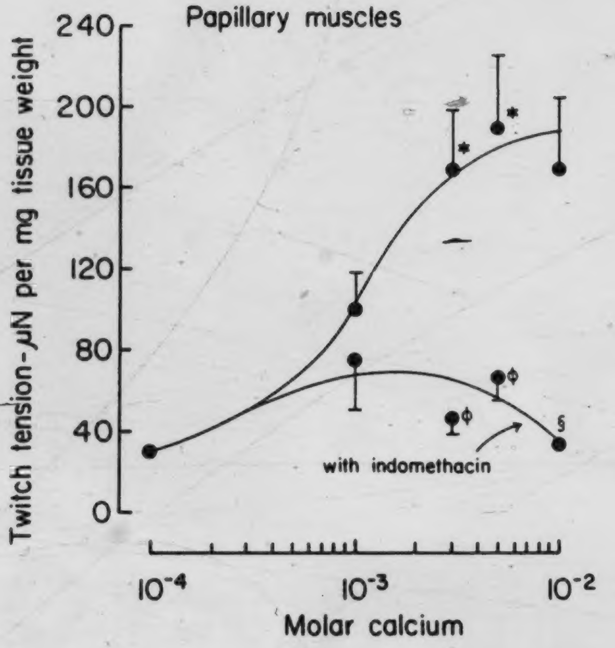


Fig. 13. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on papillary muscles from rats fed sheep fat supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 8-11; §n = 1; φn = 3).

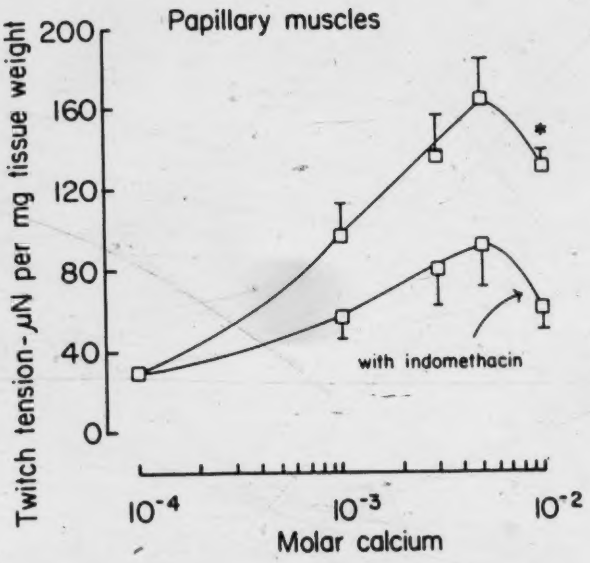


Fig. 14. Effect of 0.01 mM indomethacin on concentration-effect curve of calcium on papillary muscles from rats fed linseed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 6-14).

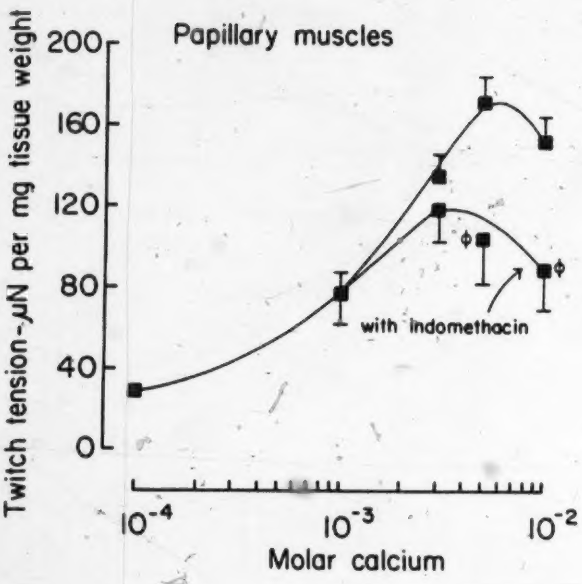


Fig. 15. Effect of 0.01 mM indomethacin concentration-effect curve of calcium on papillary muscles from rats fed sunflower seed oil supplemented diet for 9-12 weeks. No significant difference was observed between the two curves ($P>0.05$; Student's unpaired t-test). Results are mean ± S.E.M. (n = 7-13; ϕn = 3).

4.5.5 Effect of Bay K8644 on Force of Contraction in the Isolated Heart Tissues

The rat is unusual among laboratory animals in that in normal physiological solutions its cardiac tissues show little inotropic response to the presence of BAY K8644, a dihydropiridine with agonist properties at the voltage sensitive calcium channel (Finet et al. 1985). A marked positive inotropic effect can be observed if the concentration of Ca^{2+} in the bathing fluid is reduced. This, of course, reduces the control twitch tension in accordance with the slopes depicted in Fig. 4. Experiments with BAY K8644 were therefore performed in the presence of 0.75 mM Ca^{2+}.

The results obtained from atria are presented in Fig. 16, and those from papillary muscles are shown on Fig. 17. A positive inotropic effect of BAY K8644 was observed, but it was not quantitatively comparable in the different groups of atria or of papillary muscles.

In left atria, the greatest increase in twitch tension was observed in tissues isolated from the CAN-group (28 μN/mg, above a starting tension of 20 μN/mg; 140%) ($P<0.05$), followed by LIN-group (26 μN/mg, above a starting tension of 31 μN/mg; 84%) ($P<0.05$), SFO-group (19 μN/mg, above a starting tension of 39 μN/mg; 50%) ($P<0.05$), and the least increase was in tissues obtained from SKF-group (18 μN/mg, above a starting tension of 54 μN/mg; 33%) ($P<0.05$) (Fig. 16). These

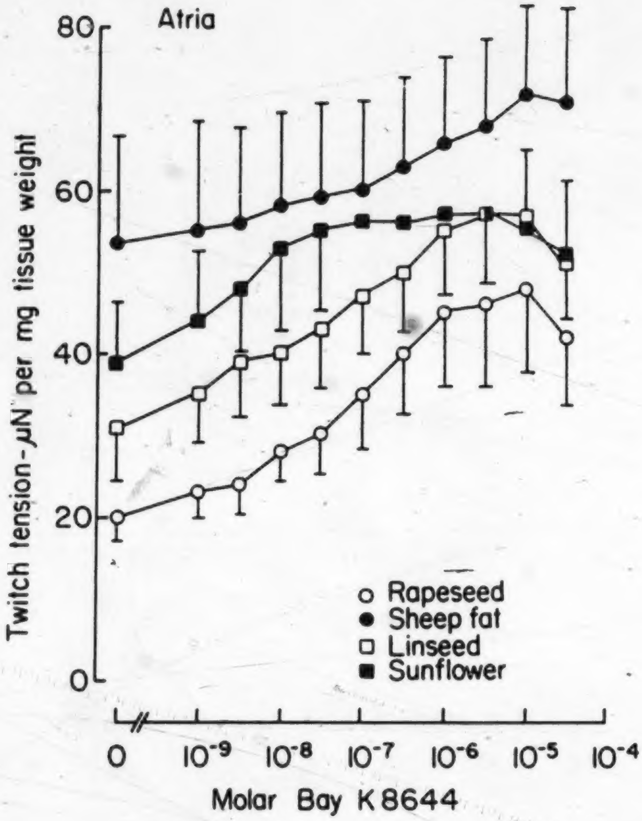


Fig. 16. Concentration-effect curves of Bay K8644 on left atria in the presence of 0.75 mM Ca^{2+}. Tissues were obtained from four groups of rats fed fat augmented diets for 9-12 weeks. Maximal increases in mean twitch tension for each group were; sheep fat, 18 μN/mg (33%) ($P<0.05$); sunflower seed oil, 19 μN/mg (50%) ($P<0.05$); linseed oil, 26 μN/mg (84%) ($P<0.05$); rapeseed oil, 28 μN/mg (140%) ($P<0.05$). Results are mean ± S.E.M. (n = 11). Points were analysed using Student's paired t-test.

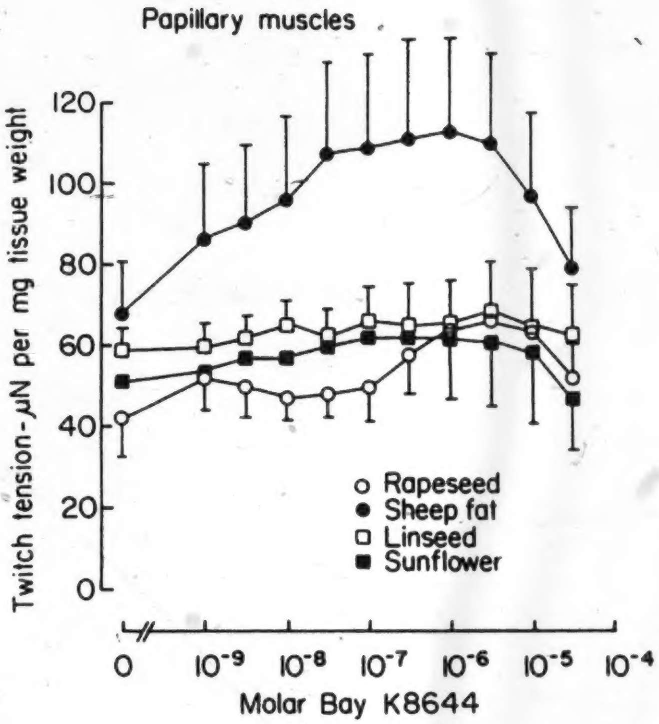


Fig. 17. Concentration-effect curves of Bay K8644 on papillary muscles in the presence of 0.75 mM Ca^{2+}. Tissues were obtained from four groups of rats fed fat augmented diets for 9-12 weeks. Maximal increases in mean twitch tension for each group were; linseed oil, 10 µN/mg (17%) (not significant); sunflower seed oil, 11 µN/mg (22%) ($P<0.05$); rapeseed oil, 24 µN/mg (57%) ($P<0.05$); sheep fat, 45 µN/mg (66%) ($P<0.05$). Results are mean ± S.E.M. (n = 8-11). Points were analysed using Student's paired t-test.

differences in responses to Bay K8644 may be partly attributed to the differing baseline twitch tension manifested by each group prior to drug addition.

Papillary muscles, on the other hand, behaved differently as the greatest inotropic response to Bay K8644 was observed in tissues from the SKF-group (45 μN/mg, above a starting tension of 68 μN/mg; 66%) ($P<0.05$), followed by CAN-group (24 μN/mg, above a starting tension of 42 μN/mg; 57%) ($P<0.05$), SFO-group (11 μN/mg, above a starting tension of 51 μN/mg; 22%) ($P<0.05$), and the least increase was in tissues obtained from the LIN-group (10 μN/mg, above a starting tension of 59 μN/mg; 17%) which was not statistically significant (Fig. 17).

4.6 SUSCEPTIBILITY OF ISOLATED HEART TISSUES FROM DIFFERENT DIETARY GROUPS TO DYSRHYTHMOGENIC STIMULI

4.6.1 Susceptibility to Dysrhythmia Induced by Ca^{2+} or Isoprenaline

During experiments on contractile tension and Ca^{2+}, it was noted that the appearance of spontaneous contractions was not common to all dietary groups. The number of tissues manifesting dysrhythmia and the threshold concentration of calcium were noted and depicted in Fig. 18. The experiment was repeated using isoprenaline as the positive inotropic stimulus in the presence of 1.53 mM Ca^{2+} (Fig. 21). This was chosen as a representative model for stress _in vitro_. Again differences in the sensitivity of tissues to the dysrhythmogenic stimulus were noted. Cumulative response-concentration curves were produced for the results to be analyzed statistically.

Left atria from CAN-group were least susceptible, either having a lower overall incidence or having a higher threshold. Atria from LIN-group shared a similar lack of susceptibility to Ca^{2+} induced dysrhythmia, but not to isoprenaline stress which caused them to manifest the highest incidence of dysrhythmia (Figs. 19 and 22).

Papillary muscles from the CAN-group also showed a lower incidence of dysrhythmia which was more evident at concentrations higher than 1 mM Ca^{2+}. On the other hand, the lower incidence with isoprenaline stress was only observed at concentrations less than 0.1 μM (Figs. 20 and 23).

4.6.2 Influence of Dietary Lipids on the Responses of Rats to Coronary Artery Ligation

It was not possible to quantify the dysrhythmia that occurred after coronary artery ligation due to the high incidence of mortality in most dietary groups. In all rats, coronary artery ligation resulted in the development of ventricular extra beats which occurred 5-10 min post-ligation. In some animals the ventricular ectopic activity progressed very rapidly into ventricular fibrillation, and when the latter did not revert to normal within 15 min the animal was considered dead. Other animals developed severe drop in mean arterial blood pressure (<30 mm Hg)—almost immediately after tying the ligature, and were considered dead when recovery did not ensue within 15 min.

After 9-12 weeks of feeding lipid supplemented diets, marked

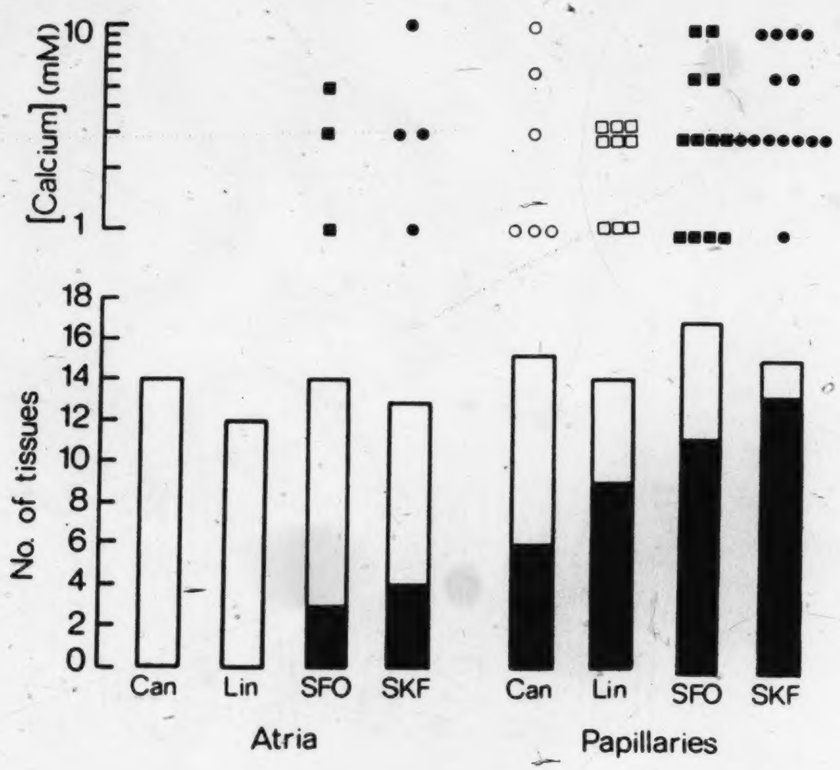


Fig. 18. Lower panel: Number of tissues (solid bar) in a dietary group (open bar) that developed dysrhythmia in response to increasing calcium concentration.

Upper panel: Threshold concentration for each tissue.

CAN = rapeseed oil; SKF = sheep fat; LIN = linseed oil; SFO = sunflower seed oil.

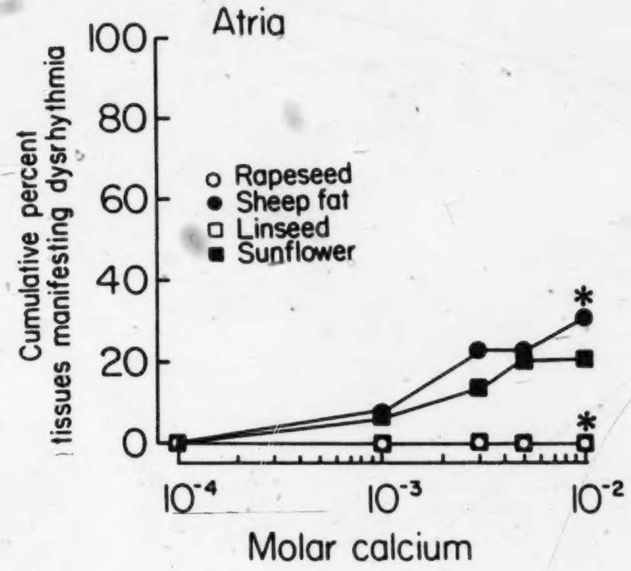


Fig. 19. Cumulative percentage of left atria developing dysrhythmia in response to increasing calcium concentration. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 months. Asterisk (*) indicates significant differences ($P<0.05$; Chi Square test or Fisher's exact probability test). n= 13-14.

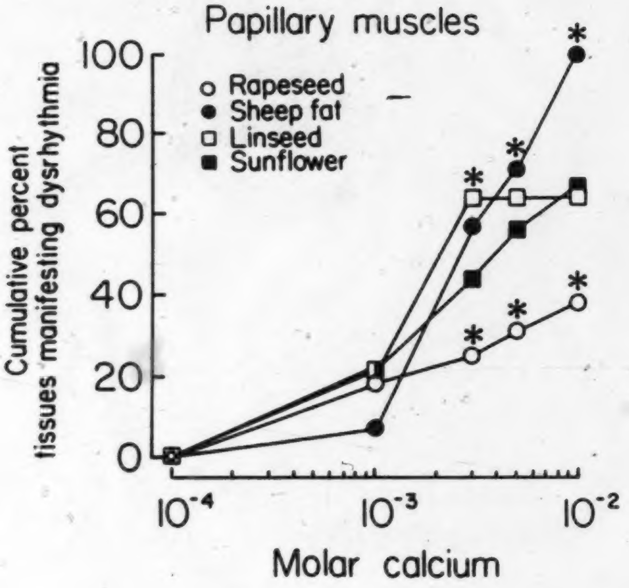


Fig. 20. Cumulative percentage of papillary muscles developing dysrhythmia in response to increasing calcium concentration. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). _n_ = 14-18.

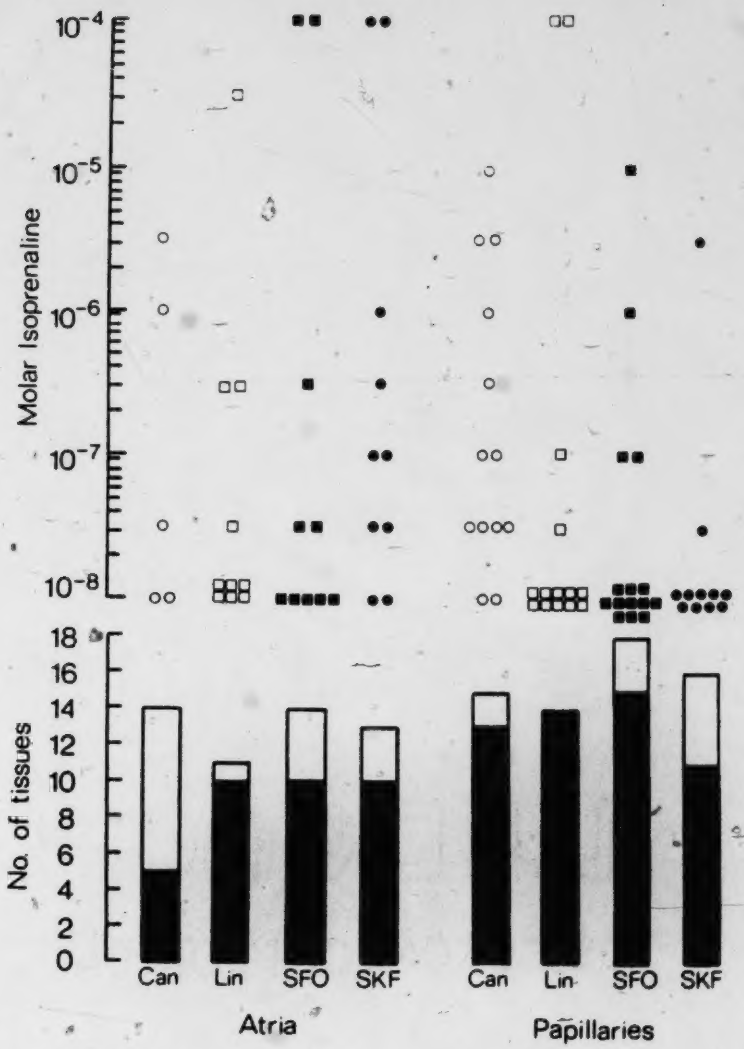


Fig. 21. Lower panel: Number of tissues (solid bar) in a dietary group (open bar) that developed dysrhythmia in response to increasing isoprenaline concentration.

Upper panel: Threshold concentration for each tissue.

CAN = rapeseed oil; SKF = sheep fat; LIN = linseed oil; SFO = sunflower seed oil.

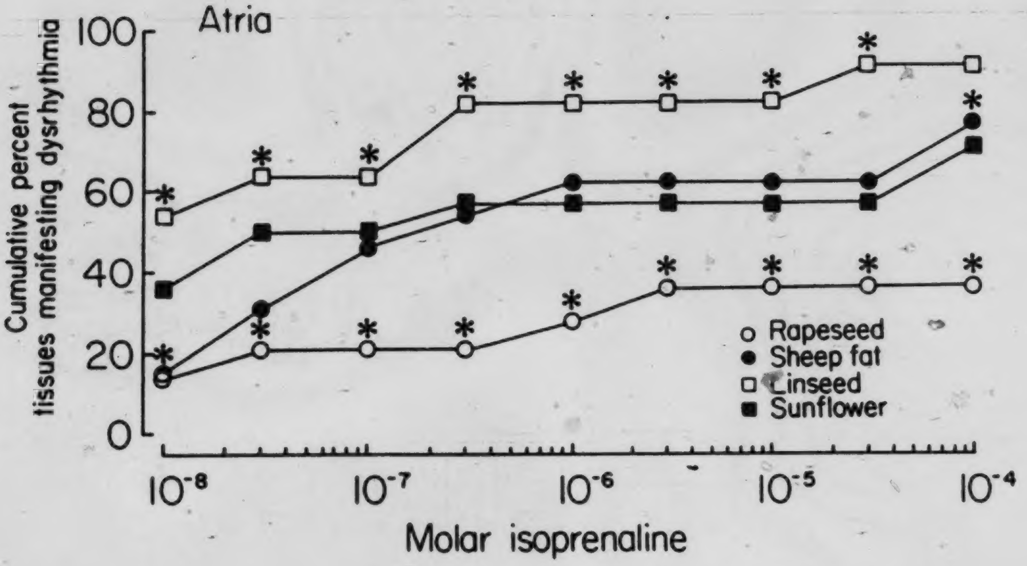


Fig. 22. Cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n=11-14.

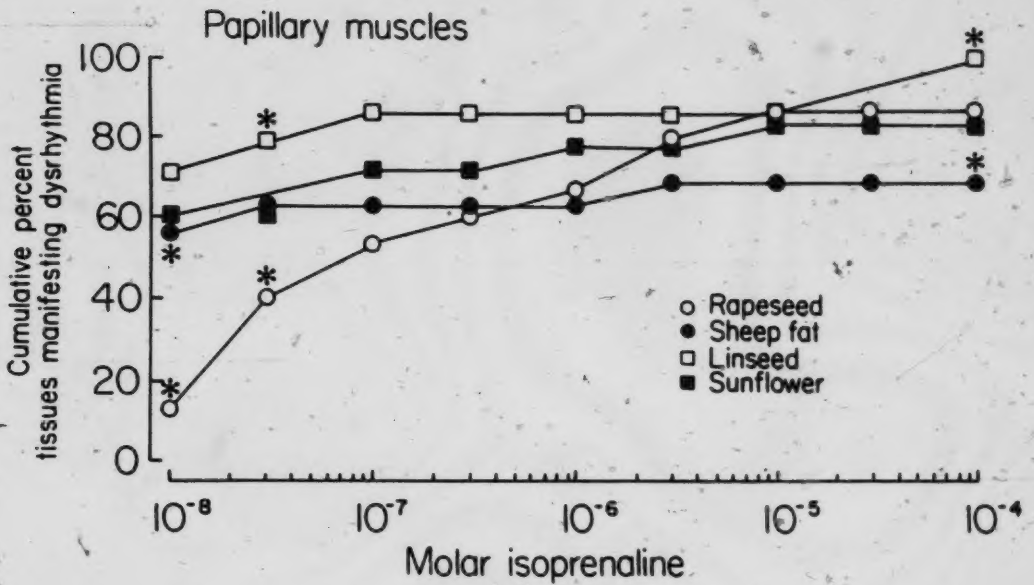


Fig. 23. Cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 months. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 14-18.

differences were found in mortality (Fig. 24). Animals in the CAN-group showed the least incidence of fatality in comparison to the other three dietary groups, and it was significantly lower than theSFO-group ($P<0.05$). A larger proportion of animals in the SFO-group died in cardiogenic shock than in ventricular fibrillation (not significant; $P>0.05$), while either cause of death had about the same incidence among the other three groups.

4.6.3 Effect of Indomethacin on Susceptibility of Isolated Heart Tissues to Dysrhythmia in the Presence of Ca^{2+} or Isoprenaline

The effect of cyclooxygenase inhibition (using 0.01 mM indomethacin) on the incidence of dysrhythmia in isolated cardiac tissues in the presence of increasing Ca^{2+} or isoprenaline concentration was observed.

While there was no significant difference between left atria from the different dietary groups (Fig. 25), none of the tissues in the SFO-group developed dysrhythmia in response to calcium in the presence of indomethacin. Tissues from SKF-group maintained a higher incidence. A similar trend was observed in papillary muscles, with the least incidence of dysrhythmia in tissues from the SFO-group which was significantly lower than SKF-group at 3 mM calcium ($P<0.05$), while at 10 mM calcium CAN-group maintained a significantly lower incidence in comparison to SKF-group ($P<0.05$) (Fig. 26).

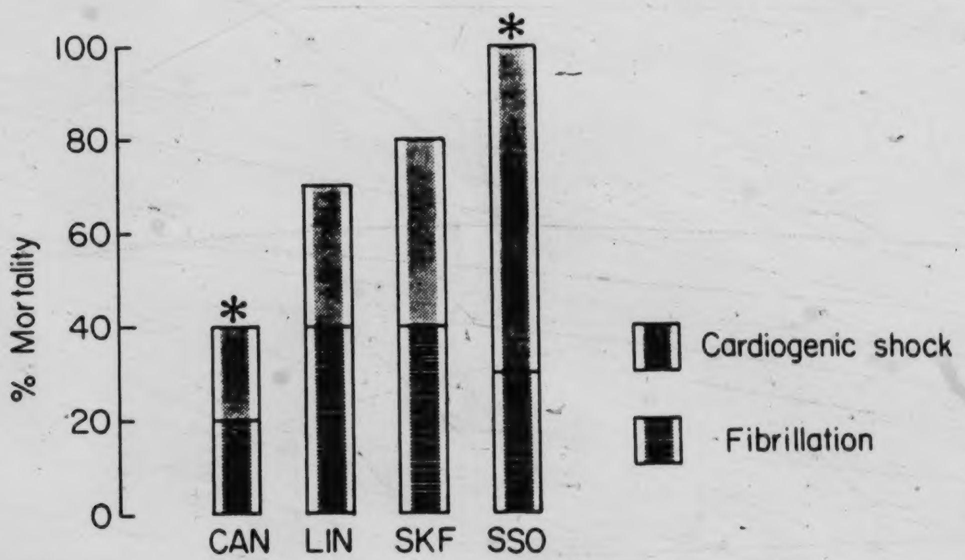


Fig. 24. Percentage of rats in each dietary group (fed for 9-12 weeks) that died within 30 min postcoronary ligation. The proportions of animals dying from either cardiogenic shock or fibrillation are as shown. Asterisk (*) indicates significant difference between the two groups ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 10 (each group). CAN = rapeseed oil; SKF = sheep fat; LIN = linseed oil; SSO = sunflower seed oil.

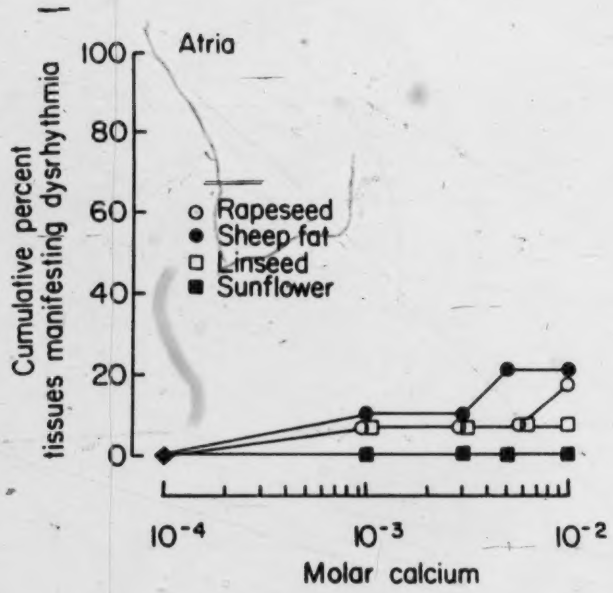


Fig. 25. Cumulative percentage of left atria developing dysrhythmia in response to increasing calcium concentration in the presence of 0.01 mM indomethacin. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Indomethacin was added 30 min prior to the addition of calcium. No significant difference was observed between the groups ($P>0.05$; Chi Square test or Fisher's exact probability test). n = 9-13.

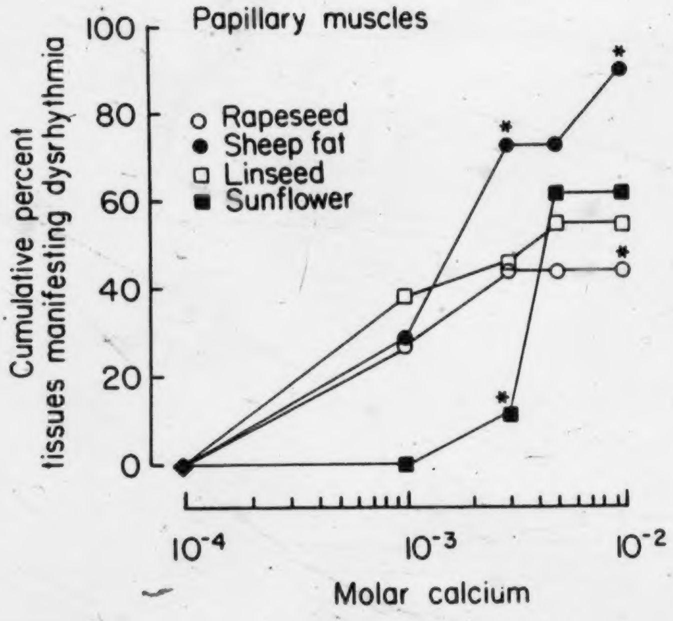


Fig. 26. Cumulative percentage of papillary muscles developing dysrhythmia in response to increasing calcium concentration in the presence of 0.01 mM indomethacin. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Indomethacin was added 30 min prior to the addition of calcium. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 8-16.

As with Ca^{2+}, no dysrhythmia was induced by isoprenaline in left atria from SFO-group in the presence of 0.01 mM indomethacin, and this was significantly different from the other dietary groups ($P<0.05$) (Fig. 27). Papillary muscles from CAN-group, on the other hand, maintained the lowest incidence of dysrhythmia at the lower concentrations of isoprenaline (0.01 μM and 0.03 μM) and the difference was significant in comparison to SKF-group ($P<0.05$) (Fig. 28). At the higher concentrations of isoprenaline (>0.1 μM) tissues from the SFO-group manifested the lowest incidence of dysrhythmia, although such a difference did not attain significance.

4.6.4 Effects of Allopurinol and Mannitol on the Susceptibility of Isolated Heart Tissues to Dysrhythmia in the Presence of Isoprenaline

The dysrhythmia pattern previously observed in left atria in the presence of isoprenaline, was changed when allopurinol (1 μM) (Fig. 29) or mannitol (20 mM) (Fig. 30) was present in the bathing medium. Atria from the SKF-group showed the highest incidence of dysrhythmia in comparison to the other three dietary groups. None of the tissues in the CAN-group developed dysrhythmia in the presence of allopurinol or mannitol, and this was significantly lower ($P<0.05$) than when only isoprenaline was present at concentrations greater than 1 μM (Fig. 31). A similar trend was observed with SKF-group, although no significant difference was detected (Fig. 32). Left atria from LIN- and SFO-groups,

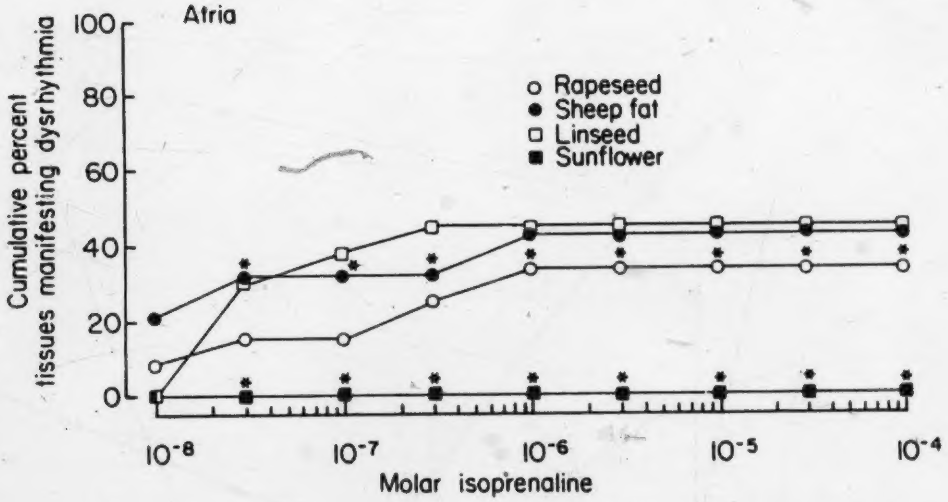


Fig. 27. Cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration in the presence of 0.01 mM indomethacin. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Indomethacin was added 30 min prior to the addition of calcium. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 9-13.

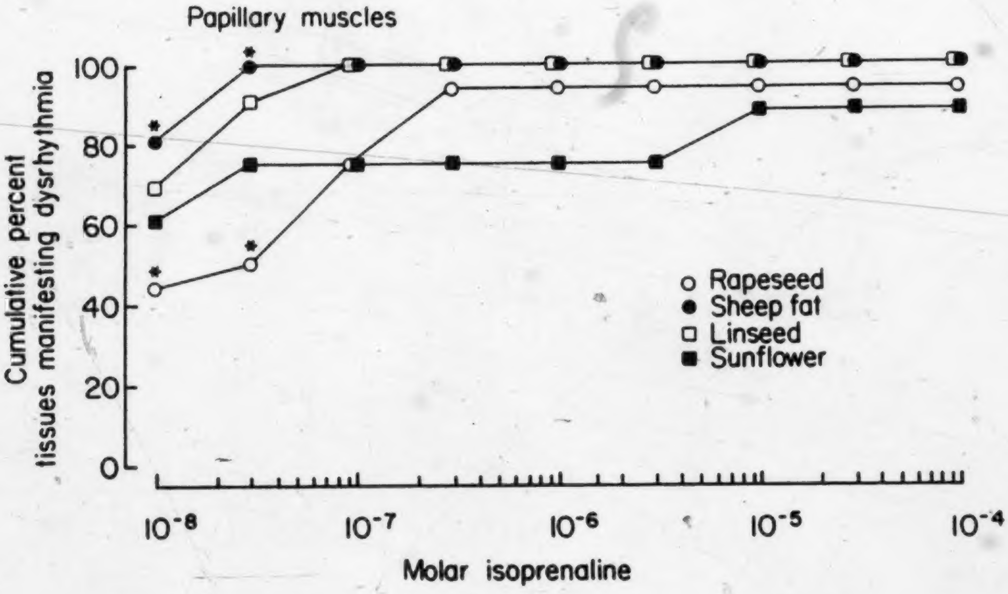


Fig. 28. Cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration in the presence of 0.01 mM indomethacin. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Indomethacin was added 30 min prior to the addition of calcium. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 8-16.

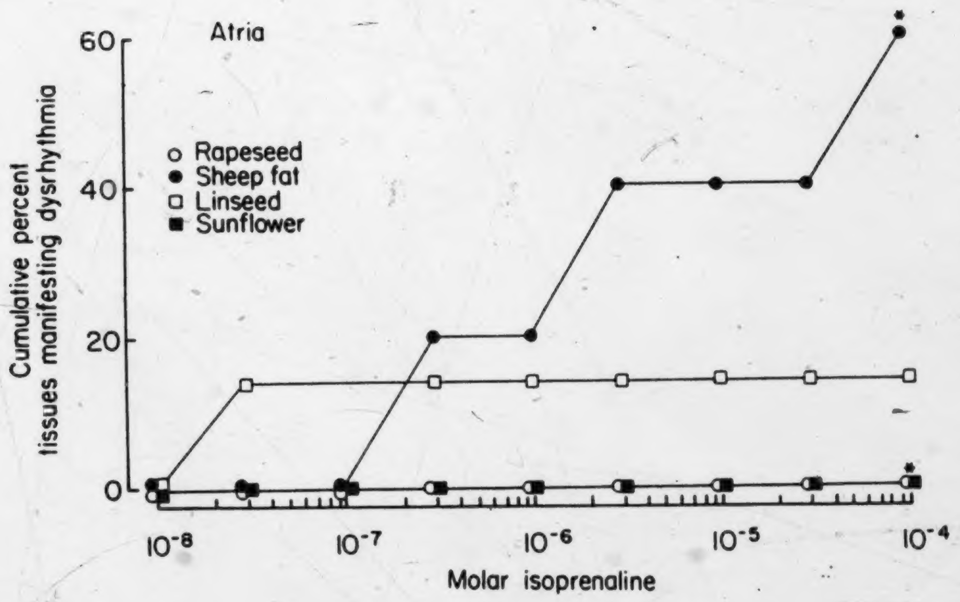


Fig. 29. Cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration in the presence of 1 μM allopurinol. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Allopurinol was added 30 min prior to the addition of isoprenaline. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 5-8.

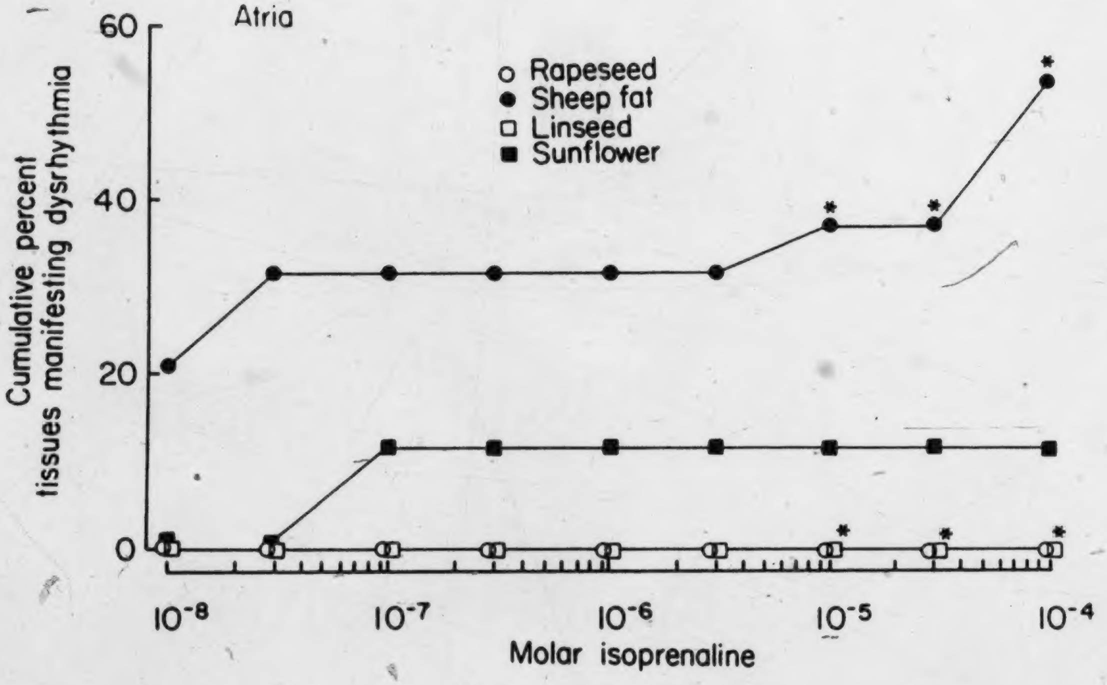


Fig. 30. Cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration in the presence of 20 mM mannitol. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Mannitol was added 30 min prior to the addition of isoprenaline. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 5-10.

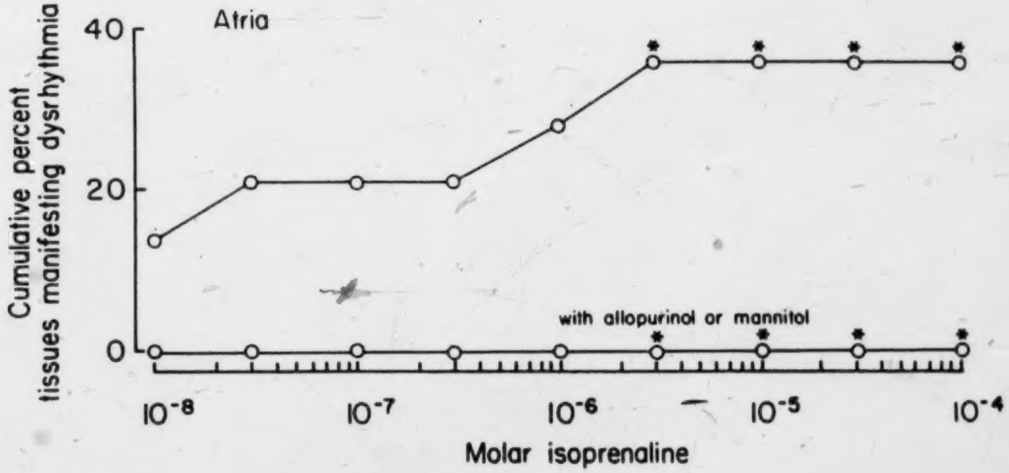


Fig. 31. Effect of allopurinol (1 µM) or mannitol (20 mM) on the cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed rapeseed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 8-14.

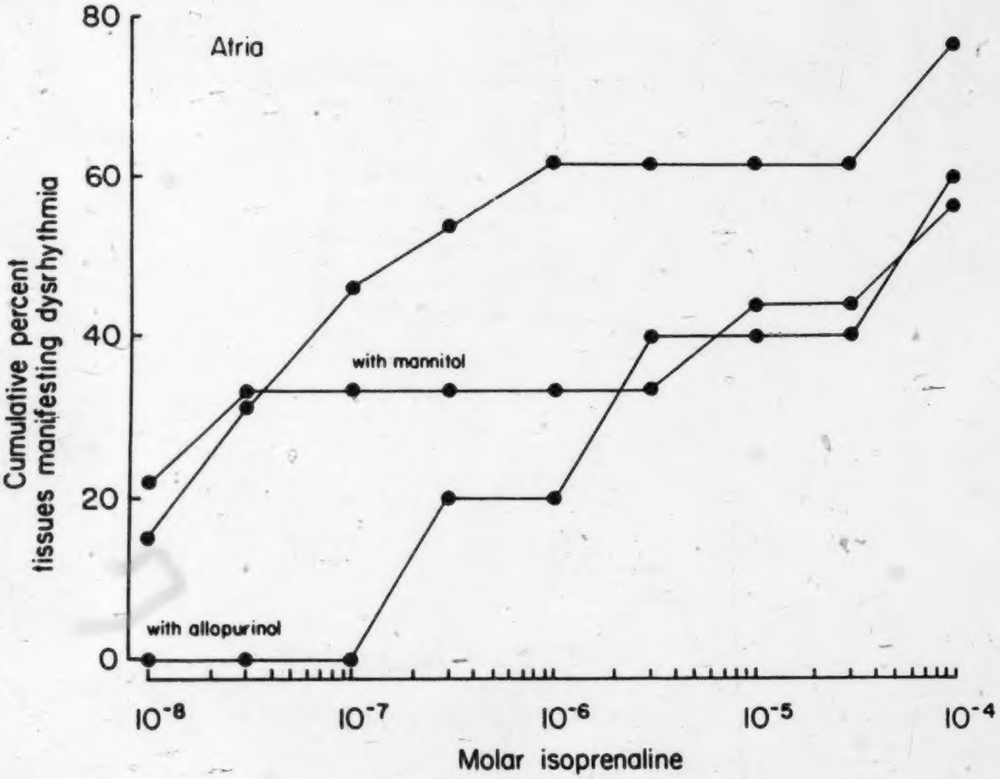


Fig. 32. Effect of allopurinol (1 µM) or mannitol (20 mM) on the cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed sheep fat supplemented diet for 9-12 weeks. No significant difference was observed between the curves ($P>0.05$; Chi Square test or Fisher's exact probability test). n = 5-13.

on the other hand, manifested a similar behavior to those from the CAN-group to increasing isoprenaline concentration in the presence of allopurinol or mannitol (Figs. 33 and 34).

In the presence of allopurinol there was no significant difference in the susceptibility of papillary muscles from any of the four dietary groups (Fig. 35). On the other hand, the incidence of dysrhythmia in the presence of mannitol was higher in papillary muscles from the SKF-group in comparison to the other three groups, and this was statistically significant ($P<0.05$) at concentrations of isoprenaline less than 1 μM (Fig. 36). When the susceptibility of papillary muscles to isoprenaline induced dysrhythmia was compared in each group in the absence and presence of allopurinol or mannitol, no significant difference was detected in all four dietary groups (Figs. 37, 38, 39 and 40).

4.7 DIETARY EFFECTS ON Ca^{2+} UPTAKE BY THE SARCOPLASMIC RETICULUM VESICLES FROM ISOLATED HEART TISSUES

The term uptake has been used to denote ATP-dependent, continuous Ca^{2+} accumulation within sarcoplasmic reticulum vesicles in the presence of a Ca^{2+}-precipitating anion, e.g. oxalate (Entman _et al_., 1973). To be able to obtain sufficient amount of S.R. fraction to perform the experiment, atria from each dietary group were pooled together, and for that reason it was not possible to perform any statistical comparison. It was obvious, however, that the rate of uptake of calcium was higher

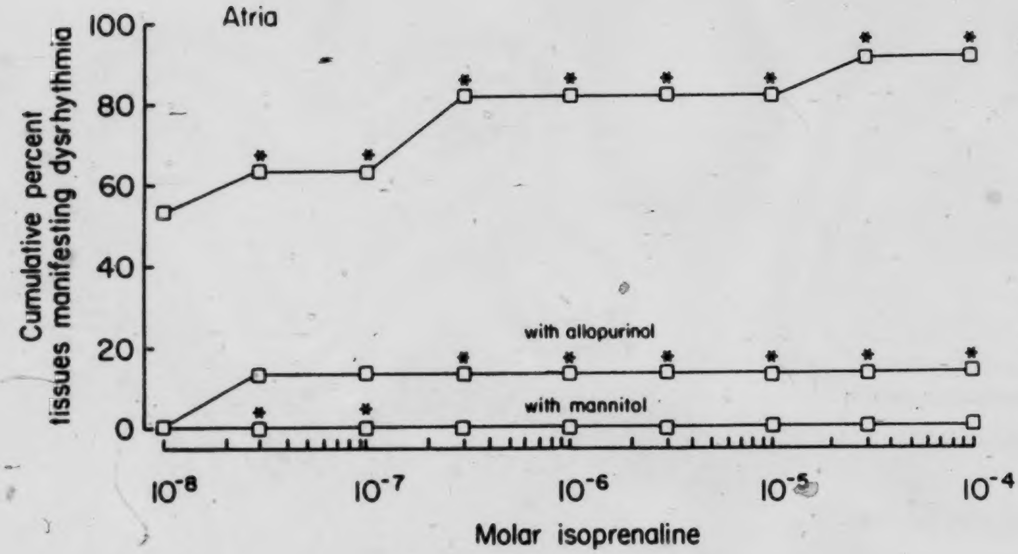


Fig. 33. Effect of allopurinol (1 μM) or mannitol (20 mM) on the cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed linseed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 5-14.

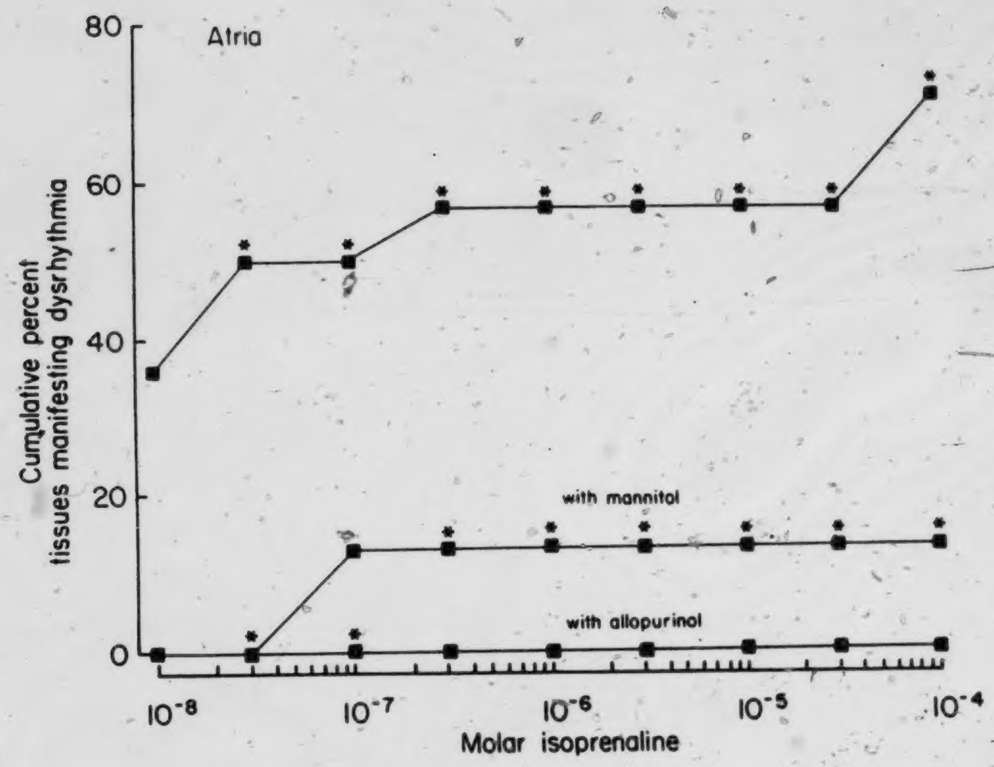


Fig. 34. Effect of allopurinol (1 μM) or mannitol (20 mM) on the cumulative percentage of left atria developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed sunflower seed oil supplemented diet for 9-12 weeks. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 8-14.

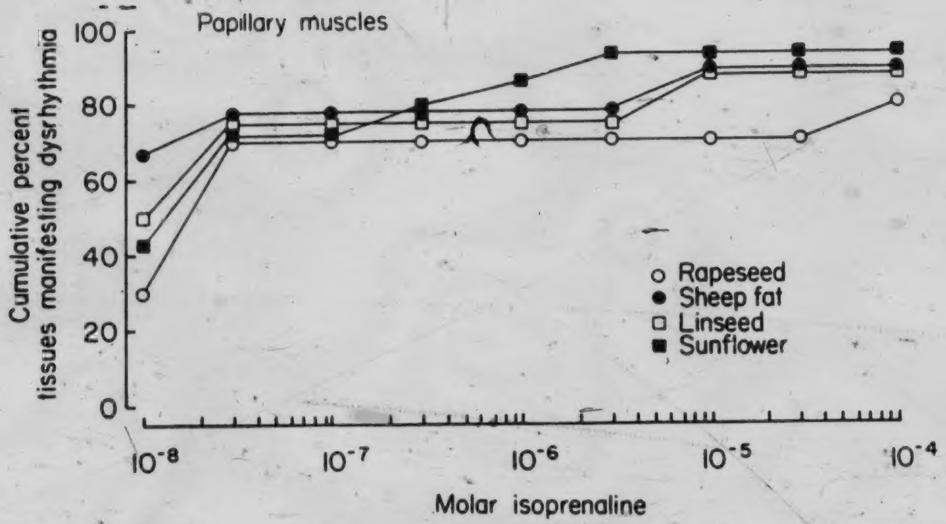


Fig. 35. Cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration in the presence of 1 μM allopurinol. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Allopurinol was added 30 min prior to the addition of isoprenaline. No significant difference was observed between the groups (P>0.05; Chi Square test or Fisher's exact probability test). n = 8-14.

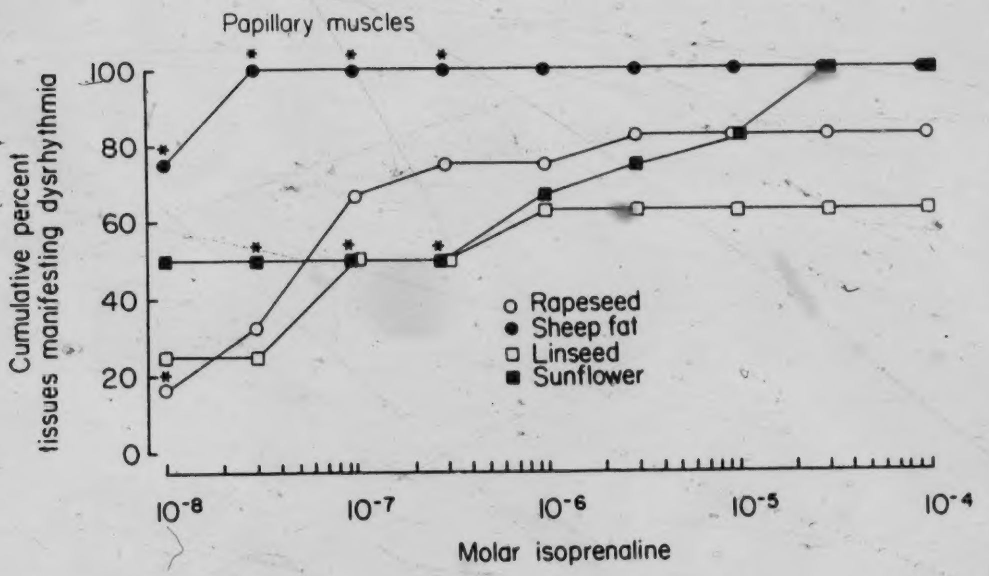


Fig. 36. Cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration in the presence of 20 mM mannitol. Tissues were isolated from four groups of rats fed fat augmented diets for 9-12 weeks. Mannitol was added 30 min prior to the addition of isoprenaline. Asterisk (*) indicates significant difference ($P<0.05$; Chi Square test or Fisher's exact probability test). n = 8-12.

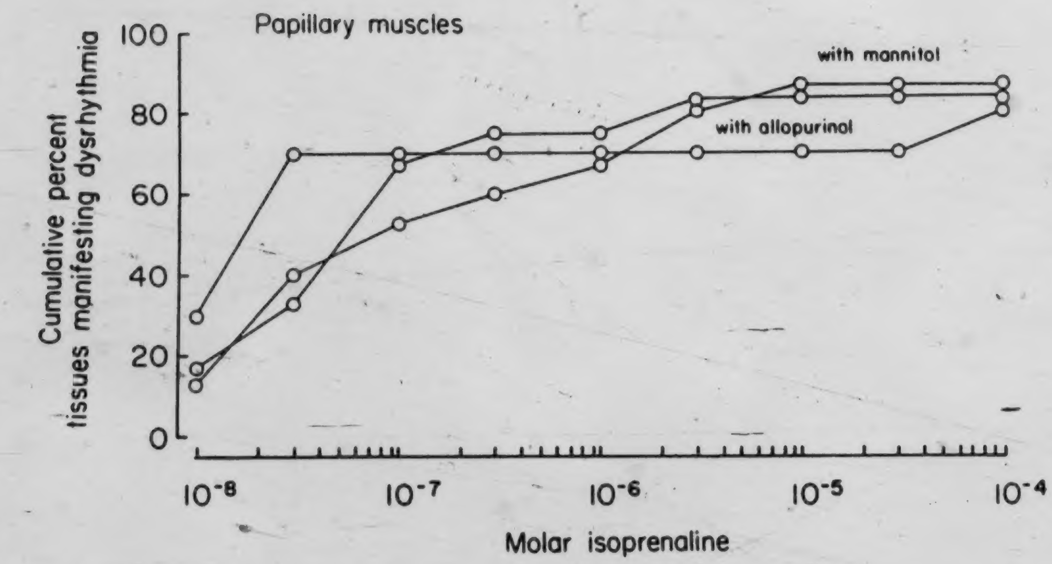


Fig. 37. Effect of allopurinol (1 μM) or mannitol (20 mM) on the cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed rapeseed oil supplemented diet for 9-12 weeks. No significant difference was observed between the curves ($P>0.05$; Chi Square test or Fisher's exact probability test). n = 9-13.

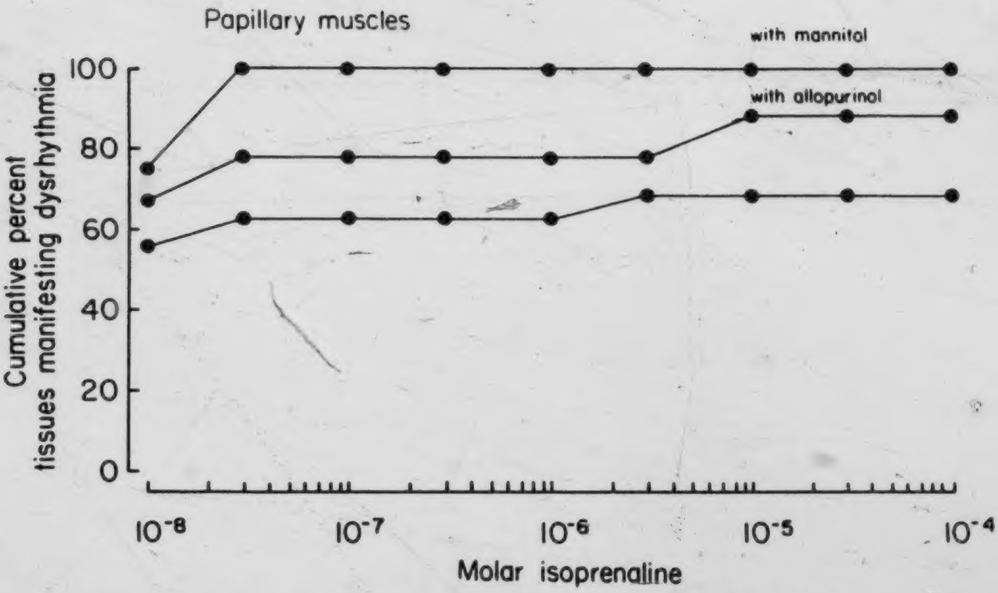


Fig. 38. Effect of allopurinol (1 μM) or mannitol (20 mM) on the cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed sheep fat supplemented diet for 9-12 weeks. No significant difference was observed between the curves ($P>0.05$; Chi Square test or Fisher's exact probability test). n = 6-11.

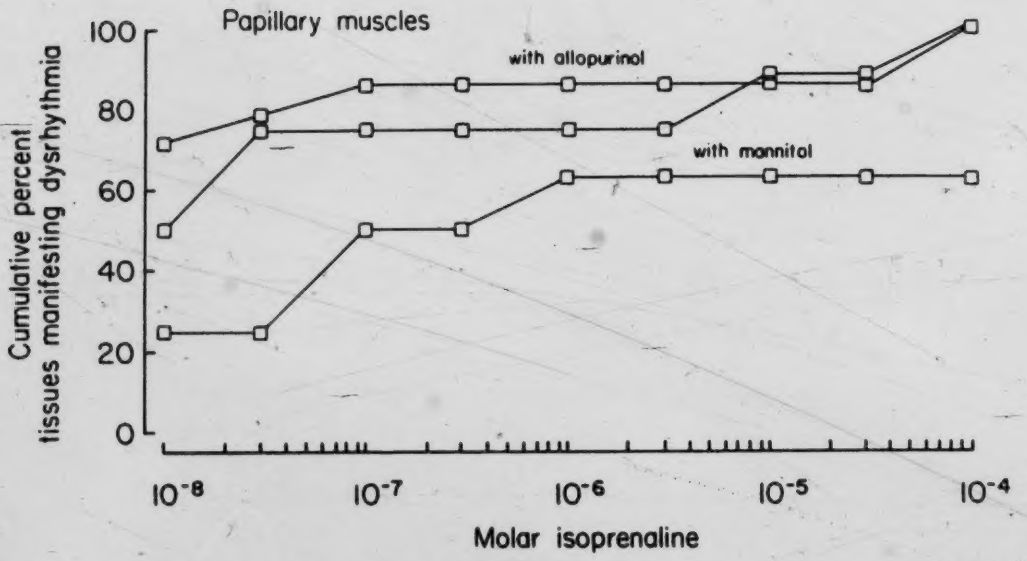


Fig. 39. Effect of allopurinol (1 μM) or mannitol (20 mM) on the cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed linseed oil supplemented diet for 9-12 weeks. No significant difference was observed between the curves (P>0.05; Chi Square test or Fisher's exact probability test). n = 8-14.

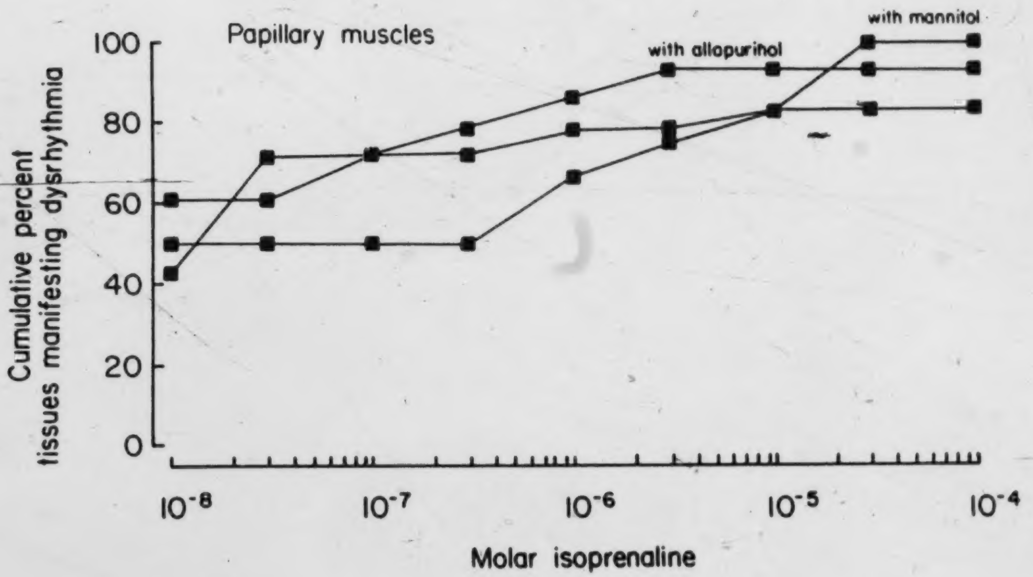


Fig. 40. Effect of allopurinol (1 μM) or mannitol (20 mM) on the cumulative percentage of papillary muscles developing dysrhythmia in response to increasing isoprenaline concentration. Tissues were isolated from rats fed sunflower seed oil supplemented diet for 9-12 weeks. No significant difference was observed between the curves ($P>0.05$; Chi Square test or Fisher's exact probability test). n = 7-14.

in fractions obtained from animals fed diets supplemented with vegetable oils in comparison to those from SKF-group, which showed a rate close to that of vesicles from animals given normal-pellet diet (Ref) (Fig. 41).

Ventricles yielded enough S.R. fraction, and thus were examined individually. The rate of uptake of Ca^{2+} by S.R. fractions from LIN-, SFO- and SKF-groups was significantly higher ($P<0.05$) than fractions obtained from either CAN- or Ref-groups which were very close together (Fig. 41).

4.8 DIETARY EFFECTS ON ATPases ACTIVITY IN SARCOPLASMIC RETICULUM VESICLES FROM ISOLATED HEART TISSUES

The activity of the total ATPase enzymes was determined concomitant with the measurement of calcium uptake by S.R. fractions from isolated heart tissues. This was carried out by estimating the release of protons during ATP hydrolysis through measurements of pH changes. Measurements were made at one-minute intervals over a period of five minutes. Figure 42 shows the hydrolysis of ATP by total ATPases of S.R. from pooled atria. One minute following the addition of S.R. fraction the activity was higher in CAN-, SKF- and LIN-groups in comparison to Ref-group, while SFO-group was intermediate. Fractions from the SKF-group hydrolyzed the greatest amount of ATP, and Ref-group the lowest over the full 5 min period. The other three groups fell in between. When averaged over the first five minutes the mean rates of

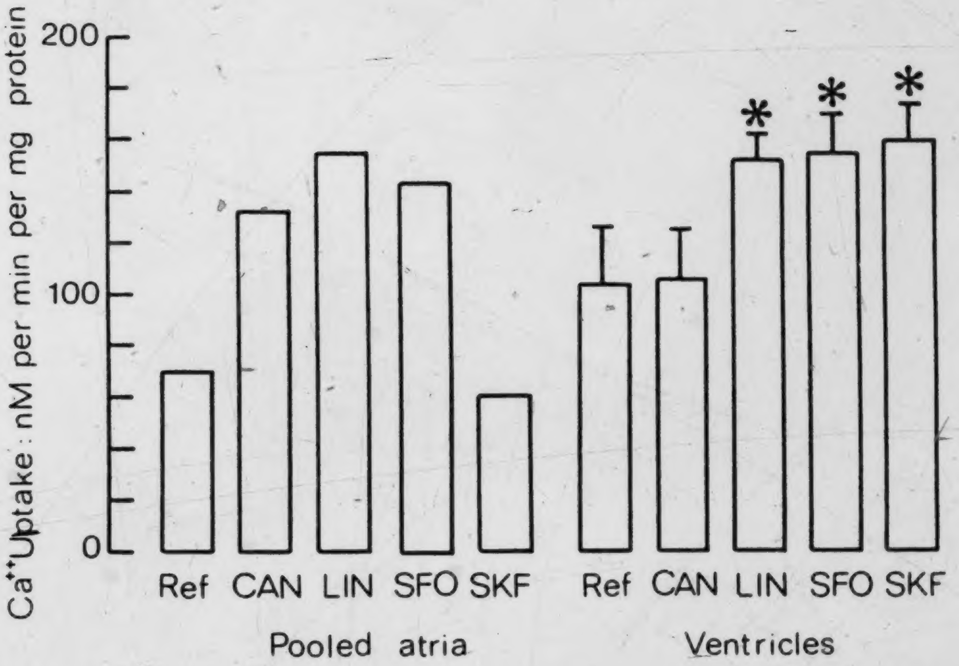


Fig. 41. Oxalate supported Ca^{2+} uptake by sarcoplasmic reticulum vesicles from atria and ventricles of rats fed normal pellets (Ref) or pellets supplemented with either rapeseed oil (CAN), sheep fat (SKF), linseed oil (LIN) or sunflower seed oil (SFO) for a period of 9-12 weeks. The reaction medium contained 4 mM Mg-ATP, 100 mM KCl, 0.5 mM $MgCl_2$, 20 mM Tris-maleate, 5 mM oxalate, 5 mM NaN_3, 100 mM sucrose, and 100 nmole Ca^{2+} at pH 7.05 and 37°C temp. Bars are mean ± S.E.M. Atria were pooled together (n=6-7) to yield sufficient S.R. fraction, while ventricular homogenates were examined individually (n=6-8). Asterisk (*) indicates significant difference from CAN and Ref. ($P<0.05$; Duncan's multiple range test).

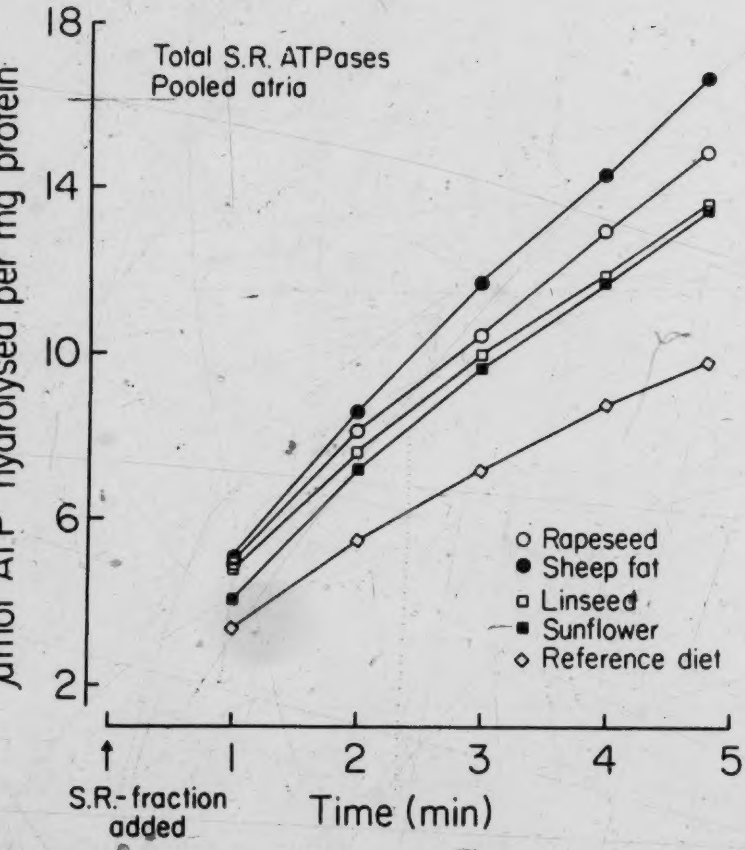


Fig. 42. Total ATPases activity of sarcoplasmic reticulum isolated from atria of rats fed either normal pellets or one of four fat augmented diets for 9-12 weeks. The reaction medium contained 4 mM Mg-ATP, 100 mM KCl, 0.5 mM $MgCl_2$, 20 mM Tris-maleate, 5 mM oxalate, 5 mM NaN_3, 100 mM sucrose, and 100 nmole Ca^{2+} at pH 7.05 and 37°C temp. Atria in each group were pooled together (n=6-7) to yield sufficient S.R. fraction.

the different fractions (μmol per mg protein per min) were: SKF, 3.34; CAN, 2.99; LIN, 2.72; SFO, 2.71; Ref, 1.96.

The activity of the Ca^{2+}-independent ATPases was measured in the absence of Ca^{2+}, but in the presence of 0.2 mM EGTA. Sarcoplasmic reticulum fractions from the LIN-group hydrolyzed the greatest amount of ATP, while the fractions from CAN-, SFO- and Ref-groups hydrolyzed least. The SKF-group was intermediate (Fig. 43). The mean activities, averaged over the first five minutes (μmol per mg protein per min) were: LIN, 2.41; SKF, 1.88; SFO, 1.72; Ref, 1.64; CAN, 1.63.

The difference in the amount of ATP hydrolyzed by the total ATPases and Ca^{2+}-independent ATPases was plotted as the hydrolysis attributed to the Ca^{2+}-dependent ATPases (Fig. 44). It was the highest in the SKF- and CAN-groups, lowest with LIN- and Ref-groups, while SFO group was intermediate. The mean activities, averaged over the first five minutes (μmol per mg protein per min) were: SKF, 1.46; CAN, 1.36; SFO, 0.99; Ref, 0.32; LIN, 0.32.

In ventricles, hydrolysis of ATP by total ATPases was significantly higher ($P<0.05$) in SKF-group in comparison to the remaining groups, 2-5 min after addition of the S.R. fraction (Fig. 45). The mean activities, averaged over the first five minutes (μmol per mg protein per min) were: SKF, 2.37; CAN, 2.07; SFO, 2.07; LIN, 1.98; Ref, 1.9.

Hydrolysis by the Ca^{2+}-independent ATPases was still significantly

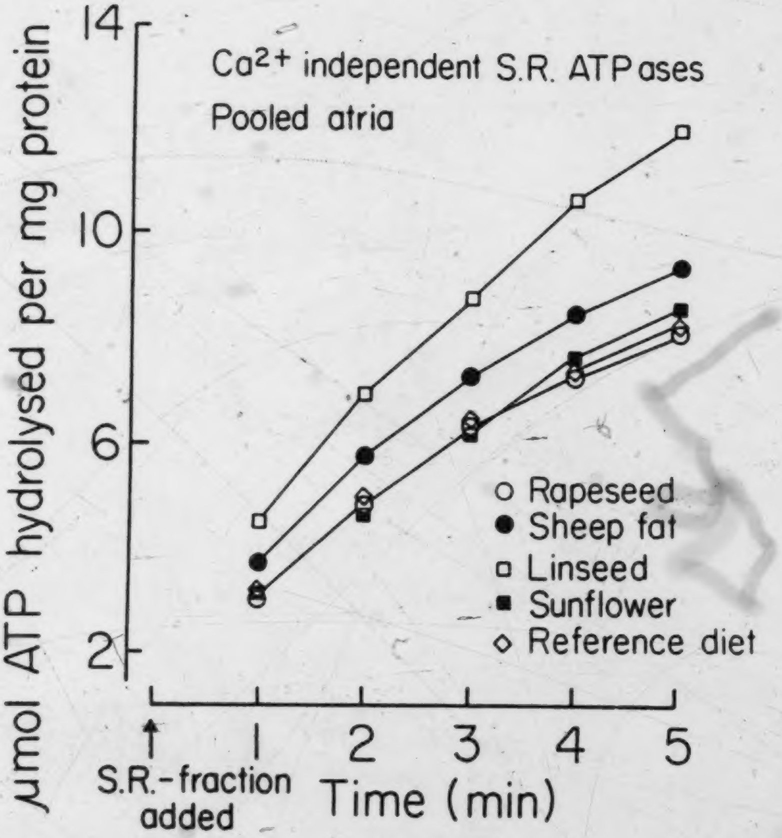


Fig. 43. Activity of Ca^{2+}-independent ATPases of sarcoplasmic reticulum isolated from atria of rats fed either normal pellets or one of four fat augmented diets for 9-12 weeks. The reaction medium contained 0.2 mM EGTA, 4 mM Mg-ATP, 100 mM KCl, 0.5 mM $MgCl_2$, 20 mM Tris-maleate, 5 mM oxalate, 5 mM NaN_3 and 100 mM sucrose at pH 7.05 and 37°C temp. Atria in each group were pooled together (n=6-7) to yield sufficient S.R. fraction.

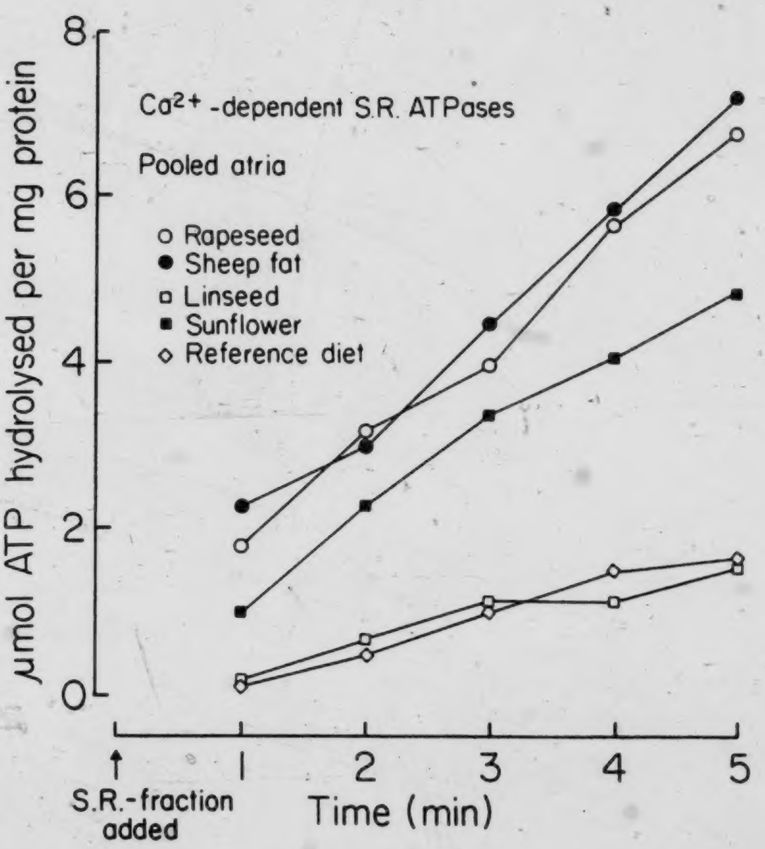


Fig. 44. Activity of Ca^{2+}-dependent ATPases of sarcoplasmic reticulum isolated from atria of rats fed either normal pellets or one of four fat augmented diets for 9-12 weeks. Each point represents the difference between the activities in the absence and presence of 0.2 mM EGTA (Figs. 43 & 44).

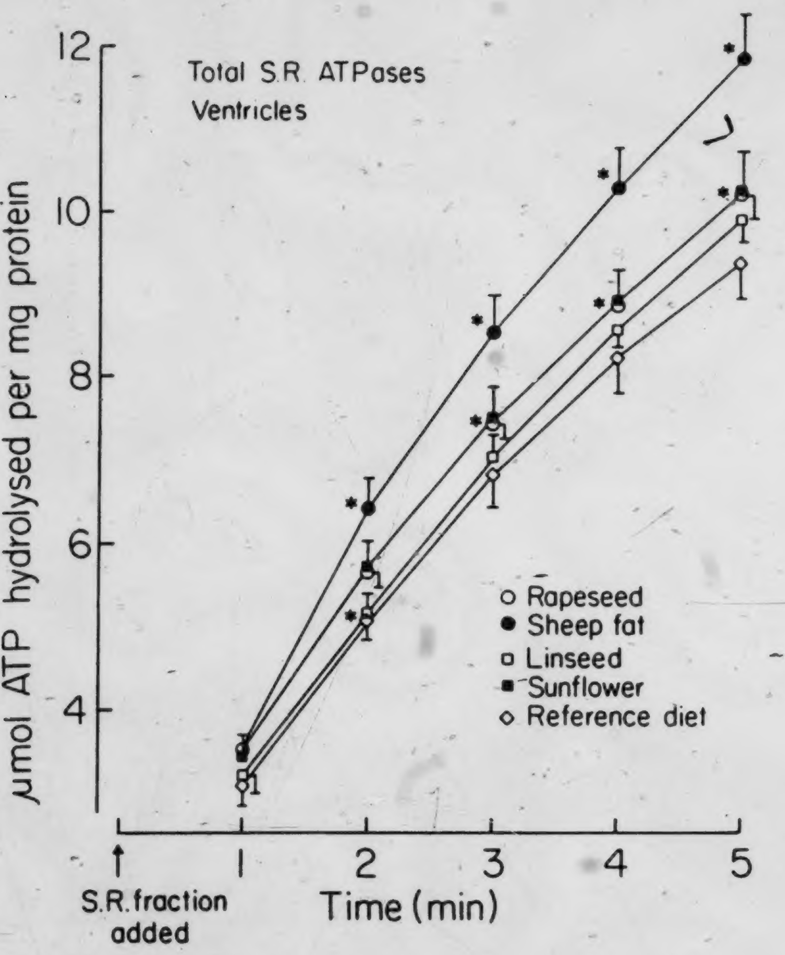


Fig. 45. Total ATPases activity of sarcoplasmic reticulum isolated from ventricles of rats fed either normal pellets or one of four fat augmented diets for 9-12 weeks. The reaction medium contained 4 mM Mg-ATP, 100 mM KCl, 0.5 mM $MgCl_2$, 20 mM Tris-maleate, 5 mM oxalate, 5 mM NaN_3, 100 mM sucrose, and 100 nmole Ca^{2+} at pH 7.05 and 37°C temp. Asterisk (*) indictes significant difference between points ($P<0.05$; Duncan's multiple range test). Points are mean ± S.E.M. (n=6-8).

greater ($P<0.05$) in SKF-group than in the remaining four groups, 1-5 min following addition of the S.R. fraction (Fig. 46). The mean activities, averaged over the first five minutes (μmol per mg protein per min) were: SKF, 1.64; Ref, 1.34; SFO, 1.33; CAN, 1.3; LIN, 1.21.

The hydrolysis of ATP by the Ca^{2+}-dependent ATPases, on the other hand, was not found to differ significantly among the five groups (Fig. 47). The mean values for activity, averaged over the first five minutes (μmol per mg protein per min) were: CAN, 0.77; LIN, 0.77; SFO, 0.74; SKF, 0.72; Ref, 0.56.

4.9 HISTOLOGICAL EXAMINATION OF LEFT VENTRICLES FROM THE DIETARY GROUPS

Histological examination of left ventricles from the four dietary groups was performed. In five out of seven tissues isolated from CAN-fed animals, grade I necrotic lesions were observed using haematoxylin-eosin stain (Plates 1 and 2). The sections were scored according to the grading system suggested by Svaar and Langmark (1980), where grade I is characterized by 1-3 small focal areas with muscle cell destruction and infiltration of histiocytes and the other chronic inflammatory cells. Only one tissue out of five examined from the SFO-dietary group manifested any lesion, which also happened to be classified as grade I (Plates 3 and 4). No lesion was detected in hearts from either LIN-group ($n=5$) or SKF-group ($n=5$).

To examine for lipidosis, sections from the same hearts were stained with Sudan black B or oil red O. A mild degree of lipidosis

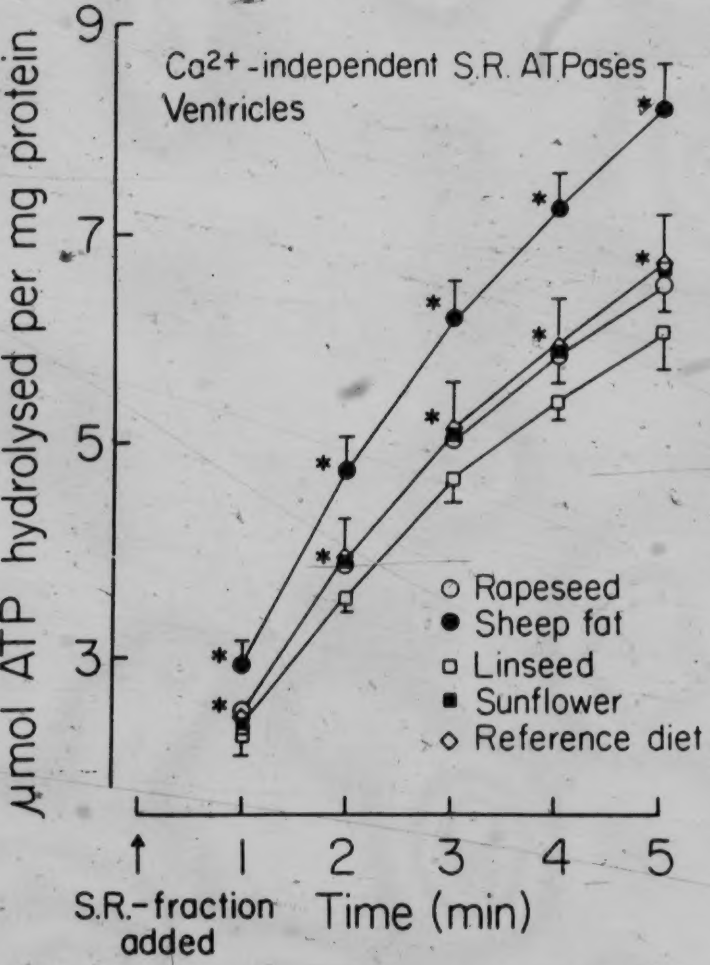


Fig. 46. Activity of Ca^{2+}-independent ATPases of sarcoplasmic reticulum isolated from ventricles of rats fed either normal pellets or one of four fat augmented diets for 9-12 weeks. The reaction medium contained 0.2 mM EGTA, 4 mM Mg-ATP, 100 mM KCl, 0.5 mM $MgCl_2$, 20 mM Tris-maleate, 5 mM oxalate, 5 mM NaN_3 and 100 mM sucrose at pH 7.05 and 37°C temp. Asterisk (*) indicates significant difference between points ($P<0.05$; Duncan's multiple range test). Points are mean ± S.E.M. (n=6-8).

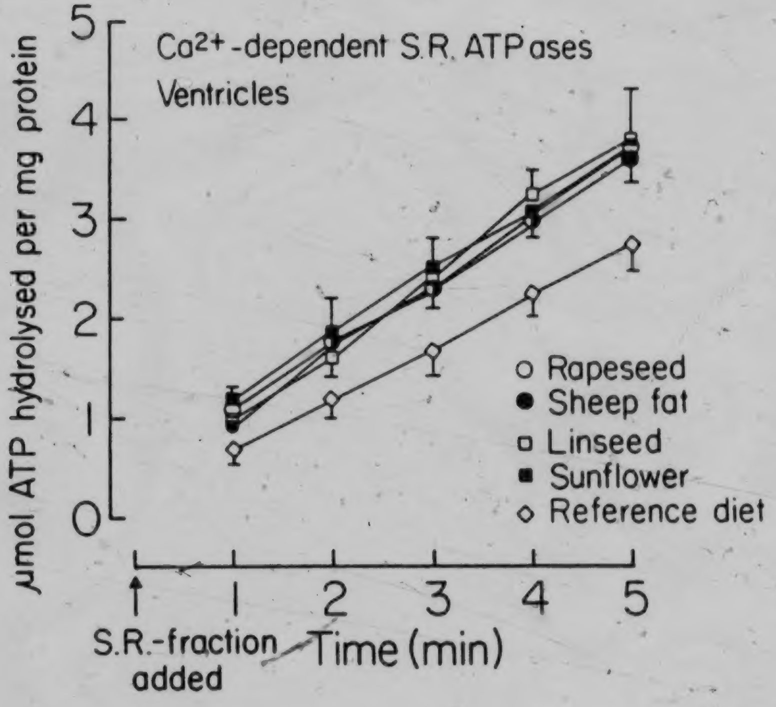


Fig. 47. Activity of Ca^{2+}-dependent ATPases of sarcoplasmic reticulum isolated from ventricles of rats fed either normal pellets or one of four fat augmented diets for 9-12 weeks. Each point represents the difference in activity in the absence and presence of 0.2 mM EGTA (Figs. 46 & 47). No significant difference was observed between the groups ($P>0.05$; Duncan's multiple range test). Points are mean + S.E.M. (n=6-8).

Plate 1. Myonecrosis and inflammatory infiltration in heart muscle.

Photomicrograph of left ventricular muscle from a rat fed rapeseed oil supplemented diet for 12 weeks. The section was stained with hematoxylin and eosin. Arrows point to area of degeneration of muscle cells and infiltration with chronic inflammatory cells. Magnification: x 240.

Plate 2. A higher magnification of the lesion in Plate 1. It shows the absence of muscle cells and replacement with histiocytes and other chronic inflammatory cells. Magnification: x 570.

Plate 3. Myonecrosis and inflammatory infiltration in heart muscle.

Photomicrograph of left ventricular muscle from a rat fed sunflower seed oil supplemented diet for 12 weeks. The section was stained with hematoxylin and eosin. Arrows point to area of degeneration of muscle cells and infiltration with chronic inflammatory cells. Magnification: x 240.

Plate 4. A higher magnification of the lesion in plate 3. It shows the absence of muscle cells and replacement with histiocytes and other chronic inflammatory cells. Magnification: x 570.

was observed in all tissues examined from the different dietary groups and are represented by Plates 5 and 6.

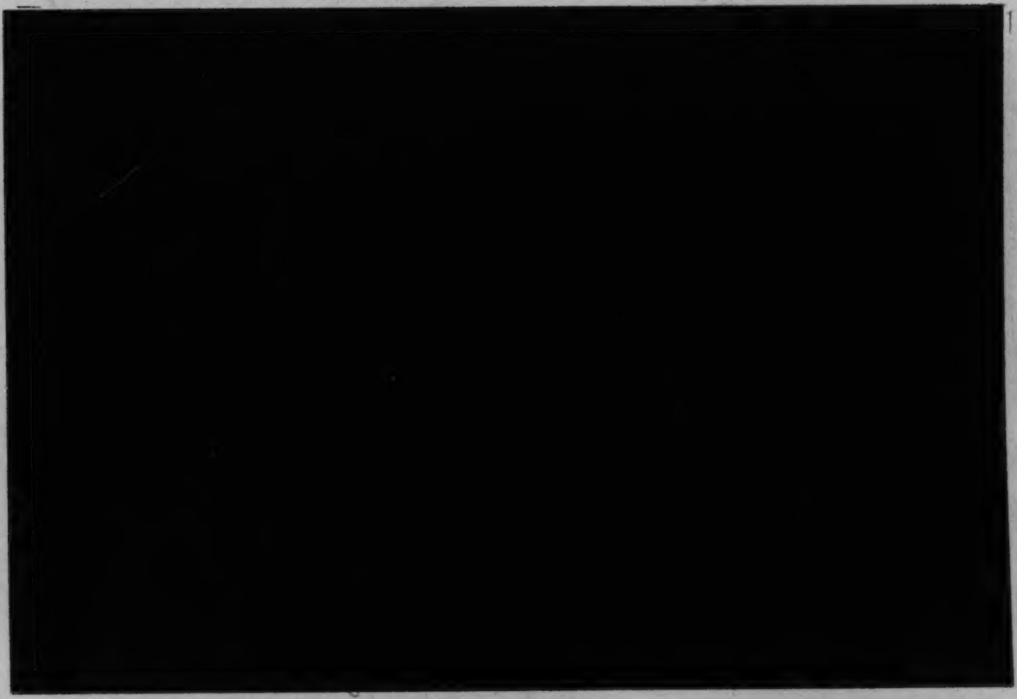


Plate 5. Mild ~~cardiac~~ lipidosis with high fat diet.

Photomicrograph of left ventricular muscle from a rat fed sheep fat supplemented diet for 12 weeks. The section was stained with oil red O. Arrows point to fat droplets inside the muscle fibres. Magnification: x 1400.

Plate 6. Mild cardiac lipidosis with high fat diet.

Photomicrograph of left ventricular muscle from a rat fed linseed oil supplemented diet for 12 weeks. The section was stained with Sudan black B. Arrows point to fat droplets inside the muscle fibre. Magnification: x 1400.

5. DISCUSSION

5.1 GENERAL

The amount of food consumed by rats in each dietary group was not accurately determined. However, diet hoppers were always depleted to about the same level over the same periods of time denoting similar consumption of different diets. This was further confirmed by the lack of significant differences in weight gained at the end of the feeding period. At the same time there was no deterioration in the health of the animals as indicated by appearance and activity. Also, no differences were observed in blood pressure and heart rate among the four groups prior to sacrifice. The conclusion must inevitably be drawn that the diets provided adequate amounts of nutrients as represented by vitamins, essential minerals, amino acids, carbohydrate and lipid. Only the proportion of fatty acids present varied, and provided the basis for the present study.

5.2 EFFECTS OF DIETARY LIPID SUPPLEMENTS ON FATTY ACID COMPOSITION OF CARDIAC PHOSPHOLIPIDS

The methods used for the analysis of tissue fatty acids are well established (section 3.8). No differences were observed in fatty acid composition, between tissues stored under liquid nitrogen, for a maximum period of 12 months, and those freshly prepared. Also, precautions taken against fatty acid oxidation during lipid extraction by using butylated hydroxy-toluene (BHT) (section 3.8.2) appeared to be successful, as no peaks from oxidised species were seen on the traces.

The results of the present work clearly indicate that differences exist in the fatty acid composition of the membrane phospholipids of atria and ventricles of the rat heart, and this is in agreement with the findings of Charnock et al. (1983).

In a later paper (Charnock et al., 1984) showed that the principal differences in the fatty acid composition of membrane phospholipids from atria and ventricles were attributable to differing amounts of individual phospholipid classes in the two tissues. Cardiolipin contributes significantly to the linoleic acid (C18:2,ω-6) content due to the high proportion of this acid present, while docosahexaenoic acid (C22:6,ω-3) is concentrated in phosphatidylcholine, and phosphatidylethanolamine.

Although the amount of saturated fatty acids available in the rats fed SKF diet was greater than that fed to either CAN-, LIN- or SFO-groups, little change was observed in the proportion of saturated fatty acids among the groups for both tissues. Nor did the ratio of saturated: unsaturated fatty acids differ markedly among the dietary groups despite the variation in the unsaturation index of the diet. The implication that this ratio is maintained by an ill-defined homeostatic mechanism is widely recognised (see Gibson et al., 1984) yet no clear explanation for this mechanism has emerged.

It is in the unsaturated fatty acid constituents that the greatest degree of variation occurs, but even here, the changes are small in comparison to the proportions of the unsaturated class precursors present in the diet (Tables 1, 4 and 5). The unsaturation index which is broadly related to membrane fluidity (Katz and Messineo, 1981a and b; Stubbs and Smith, 1984; Spector and Yorek, 1985), is kept within relatively narrow bounds. This is achieved, apparently by incorporating acids of the ω-6 series where they are abundant in the SFO diet, but where their proportion is reduced, acids of both ω-9 (monounsaturated) and ω-3 appear in compensatory higher proportions. This reciprocal relationship between the proportions of ω-6 and ω-3 acids has been commented on by others (Kramer, 1980; Tahin *et al.*, 1981; De Schryver and Privett, 1982; Gibsen *et al.*, 1984; Charnock *et al.*, 1985a). The present work draws attention to the combined role of both ω-9 and ω-3 acids in maintaining the unsaturation index. Even so the Σω6 value shows only modest variation among membrane phospholipids of the SKF-, CAN- and LIN-groups despite variations in the dietary supply either as a percent of total fatty acids or of unsaturated fatty acids.

As others have noted (Kramer, Farnworth and Thompson, 1985) the proportion of arachidonic acid in membrane phospholipids is remarkably constant, reflecting, perhaps, the important role that this acid plays as a precursor of pharmacologically active eicosanoids. Variation in the ω-6 group is largely caused by varying the linoleic acid (C18:2) content of the membranes, and by different amounts of the products of elongation of arachidonic acid namely docosatetraenoic (C22:4,ω-6) and docosapentaenoic (C22:5,ω-6) acids.

A further paradox is that the proportions of the ω-9 and ω-3 acids that do appear in the membranes are not always in keeping with their proportions in the diet. In the sheep fat supplemented diet oleic acid (C18:1,ω-9) comprises 88.4% of all unsaturated acids, yet the proportion of oleic acid in membrane phospholipids is appreciably less than in tissues from animals fed a rapeseed supplemented diet where oleic acid is 64% of all unsaturated acids. Sheep fat diet contains only 0.7% ω-3 acids (present as linolenate) (1.3% of unsaturated acids) yet the membranes of animals fed this diet contain the highest proportion of ω-3 acids (present largely as docosahexaenoic acid).

It is clear, therefore, that the simple elongation and desaturation of substrates by the elongase system or Δ^6 desaturase on the basis of substrate specificity as described by Cook (1982) and Brenner and Peluffo (1966) and the proportion of substrates provided by the diets is insufficient to explain the ultimate composition of phospholipids. Nor can mutual substrate inhibition of the three fatty acid metabolic pathways by the dietary supplies of the progenitor acids as described by Mohrhauer and Holman (1963) explain the anomalies seen when tissues derived from animals fed the four diets used in the present study are compared.

A more complex series of controls, as suggested by Naughton (1981), must be postulated to explain the apparent homeostasis of the saturate/unsaturate ratio, arachidonic acid content, unsaturation index, and by extension, probably membrane fluidity noted in the present study. An examination of the individual phospholipids present might provide greater insight on this possibility.

5.3 EFFECTS OF DIETARY LIPID SUPPLEMENTS ON CARDIAC CONTRACTILITY

Previous reports have suggested that dietary lipids might affect cardiac contractility (De Deckere and Ten Hoor, 1979, 1980; Hoffman *et al*., 1982) but these workers used the isolated perfused whole heart as the experimental model. While such models are valuable, interpretation of the data with respect to contractility is rendered difficult because of varying chronotropic and perfusion characteristics inherent in the system. It is not certain, for instance, to what extent the increased force of contraction seen by Hoffman *et al*. (1982) in hearts from animals fed linoleic acid rich diets was attributable to the reduced rate of spontaneous contraction in the same tissues since frequency of contraction is itself an inotropic factor. De Deckere and Ten Hoor (1980) in similar work noted that the spontaneous frequencies of hearts from sunflower seed oil fed animals were higher than those from lard fed animals. Despite this the left ventricular work output was greater in the sunflower group when hearts were driven at a constant rate. The observed increased coronary flow would of course provide greater oxygenation to the myocardium and thus increase work capacity. The present study has concentrated on the isolated tissues from the heart in an attempt to avoid the complications of variable frequencies and coronary flow, and to investigate the different responses of atrial and papillary muscles.

5.3.1 Dietary Effects on Length-Tension Relation

The relation between muscle length or sarcomere length and developed tension for lengths up to the optimal for contraction (L_{max}) is much steeper in cardiac muscle than in skeletal muscle (Gordon _et al_., 1966; Brutsaert and Sonnenblick, 1969; Forman _et al_., 1972). The steepness of the cardiac length-tension relation arises because the degree of activation of the cardiac myofibrils by Ca^{2+} increases as muscle length is increased (Jewell, 1977). Allen and Kentish (1985) suggested that two processes contribute to this length-dependence of activation: (i) the Ca^{2+} sensitivity of the myofibrils increases with muscle length and (ii) the amount of Ca^{2+} supplied to the myofibrils during systole increases with muscle length. Of these two, the change in Ca^{2+} sensitivity is the most clearly defined and is responsible for a large part of the rapid change in developed tension when muscle length is altered. It is likely that this change in Ca^{2+} sensitivity is due to a change in the affinity of troponin for Ca^{2+} (Endo, 1972, Fabiato and Fabiato, 1978; Moss _et al_., 1983), but the underlying mechanism has not been identified. A change in the Ca^{2+} supply to the myofibrils with change in length is less clearly established and is still controversial.

The results obtained in this study showed no alteration in length-tension relationship of atria and papillary muscles from the four dietary groups, suggesting a lack of effect of dietary lipids on the sensitivity of the contractile elements to Ca^{2+}.

5.3.2 Dietary Effects on Contractile Responses to Ca^{2+}

Ten Hoor et al. (1973) noted that an absolute deficiency of linoleic acid in the diet caused a profound reduction in the tension and contractility of isolated papillary muscles over a six month period. In the present study, however, the four diets contained adequate levels of linoleic acid. Our results indicate a differential inotropic response between atria and ventricles towards Ca^{2+} after 9-12 weeks of dietary supplementation. The finding that tissues from CAN-group manifested the lowest tension, is supported by the results of De Wildt and Speijers (1984). They observed less contractile reserve capacity, after positive inotropic intervention, in hearts of animals fed rapeseed oil in comparison with animals fed either SFO or erucic acid supplemented diet. This led to the conclusion that the decrease in contractility could not be attributed to erucic acid, which was also absent in the rapeseed oil utilized in the present study.

Precisely how external calcium is capable of affecting the force developed during a twitch tension is a matter of debate (Fabiato and Fabiato, 1979a), as is the relative contribution of extra - and intracellular Ca^{2+} to contractile activation. It certainly varies among species, with the most extreme case being the adult rat heart where Ca^{2+} is mainly derived from intracellular stores (Fabiato and Fabiato, 1978a; Vornanen, 1984). However, there is general agreement that there are at least two stages for Ca^{2+} involvement in force development. The first stage involves a transsarcolemmal movement of

C[illegible] associated principally with the plateau of the action potential (Langer, 1976, 1984). The second stage involves the release into the myoplasm of Ca^{2+} held in sarcotubular stores and is dependent on the first stage (Langer, 1980; Chapman, 1983). That is to say that the tension developed during the twitch is a function of the amount of Ca^{2+} crossing the sarcolemma during the excitation of the cell. The Ca^{2+} available for this purpose is currently believed to be bound in part to anionic phospholipids of the sarcolemma and also to the glycocalyx (Burt _et_ _al_., 1984; Langers, 1984; Borgers _et_ _al_., 1985) and is sensitive to external Ca^{2+} levels (Kitazawa, 1984). Since the proportions of the various major classes of phospholipids in membranes are unaffected by dietary lipid variation (Kramer, 1980; Charnock _et_ _al_., 1984), Ca^{2+} binding to membranes on account of phospholipid proportions cannot be altered. However, although Ca^{2+} binding to each phospholipid molecule occurs at the anionic head group, the extent of binding is inversely affected by the degree of unsaturation of the constituent acyl chains (Holub and Kuksis, 1978). Although the composition of sarcolemmal phospholipids was not determined separately in the present study the possibility that the binding of Ca^{2+} to the sarcolemma is changed by changes in the fatty acid composition of the sarcolemmal phospholipids cannot be rigorously excluded as an explanation for the difference in contractility observed. Hitherto the discussion has centred on fatty acids in membrane phospholipids. However, free fatty acids also exist inside cells and may have effects noticeable in contractility studies. Lammers and Hulsmann (1977) noted that free fatty acids caused inhibition of $(Na^+ + K^+)$ATPase; a biochemical intervention that normally causes a positive inotropic response in the intact beating heart. Using purified cardiac sarcolemmal vesicles, Ashavid et al. (1985) studied the effects of two fatty acids on Na^+ dependent Ca^{2+} uptake. Palmitic acid was found to inhibit, whereas oleic acid stimulated initial Ca^{2+} uptake without disruption of membrane vesicle structure. Munkonge et al. (1985) noted that oleic acid, but not palmitic acid, could uncouple Ca^{2+} accumulation from the catalytic activity of Ca^{2+}-ATPase in vesicles of sarcoplasmic reticulum and at higher concentrations (2μmol per mg membrane protein) completely inhibit the enzyme. This observation was further supported by the findings of Utsumi et al. (1985) who reported an enhancement of Ca^{2+} translocation across liposomal membranes, from the outside to the inside, by unsaturated long chain fatty acids; oleic, linoleic, linolenic and arachidonic. Such an effect was not observed with saturated fatty acids. Due to the fact that the potency of the various fatty acids in the stimulation of Ca^{2+} transport was inversely correlated with their melting points, it was suggested that these fatty acids might alter some of the physical properties of membranes, e.g. fluidity, which in turn can influence the activity of certain membrane bound enzymes as $(Na^+ + K^+)$-ATPase (Abeywardena et al., 1983), or affect in some way the protein channels resulting in an alteration of trans-membrane ion movements (Katz and Messineo, 1981a).

A relationship between phospholipid acyl chain unsaturation and fluidity is supported by studies of model and natural membranes. Cis-unsaturation of phospholipid acyl chains increases the molecular packing areas in monolayers and bilayer liposomes (Demel et al., 1972) and enhances the fluidity of model membranes (Lentz et al., 1976; Seelig and Seelig, 1977). Changes in the fluidity of biological membranes owing to alterations in the content of unsaturated acyl chains have been observed by a number of investigators (Kasai et al., 1976; King et al., 1977; King and Spector, 1978; Storch and Schachter, 1984) but not by others (Stubbs et al., 1980; McVey et al., 1981; Poon et al., 1981; McMurchie et al., 1983c). Abeywardena et al. (1984) provided convincing evidence that dietary alteration of membrane phospholipids had no effect on the critical temperature of Arrhenius plots of sarcolemma ($Na^+ + K^+$)ATPase, sarcoplasmic reticulum Ca^{2+}-ATPase or mitochondrial F_1-ATPase, nor on polarization fluorimetry of these membranes and so an effect on phospholipid fluidity may be discounted. Although free oleic acid can affect membrane fluidity at concentrations of 1μmol per mg protein (Munkonge et al. 1985), and it is assumed that this effect extends to other more unsaturated fatty acids, it is not known if the concentration of free fatty acids available in the experimental tissues could account for changes in contractility by this mechanism. The subject of free fatty acid concentrations in heart tissues is controversial (Victor et al., 1984; Van der Vusse et al. 1986). Although concentrations in vivo are probably too low for significant intercalation into membrane phospholipids, in the marginally hypoxic conditions of in vitro preparations these concentrations may rise and affect the contractility of muscle (Henderson et al. 1970; Dryden - personal communication). Thus the possibility exists that inotropic differences may be ascribed to free fatty acid generation in the conditions of the experiments described.

Inositol phospholipids have recently been recognized as an additional system capable of acting as second messengers in a wide variety of cell types (Berridge & Irvine, 1984; Hirasawa & Nishizuka, 1985). Although the complete sequence of events is not at present known, the steps involved in signal transduction involve the release of inositol 1,4,5,-triphosphate and diacylglycerol from the membrane embedded phospholipid (Berridge, 1984). Inositol 1,4,5-triphosphate (ITP) has been postulated to release Ca^{2+} from intracellular stores (Michell, 1975; Berridge, 1983; Streb et al., 1983; Burgess, 1984; Joseph et al., 1984) including those in skeletal muscle (Vergara et al., 1985). In addition the Ca^{2+} sensitivity of chemically skinned skeletal muscle fibres is enhanced by exogenous ITP (Thieleczek and Heilmeyer, 1986). Diacylglycerol (DAG), on the other hand, is believed to activate protein kinase C and in turn modulate cell function (Takai et al., 1979; Kishimoto et al., 1980; Kaibuchi et al., 1982; Takai et al., 1982; Nishizuka, 1983).

There is recent evidence that cardiac tissue is also sensitive to a phosphatidyl inositol mediated messenger system (Knabb et al., 1984).

Phosphatidylinositol breakdown may be compromised by either varying the fatty acid composition of the surrounding membrane phospholipids, or the fatty acid composition of phosphatidylinositol itself following dietary pressures (Van Rooijen et al., 1985). It is not known if diacylglycerols of differing fatty acid composition exert qualitatively or quantitatively different responses, or if phospholipase C activity is affected by the DAG composition in the substrate phosphatidyl inositol. It is possible to postulate an effect of dietary lipid mediated either through different amounts of ITP release or different effects of the released DAG. Again this presents an area for further work.

5.3.2.1 Do dietary lipid supplements modify myofibrillar responsiveness to Ca^{2+}?

One of the most straightforward ways of obtaining evidence about how an intervention alters the myofibrillar response to Ca^{2+} is through the use of "skinned" or "hyperpermeable" muscle fiber preparations, in which the relationship between Ca^{2+} and tension development can be explored directly.

Treating left atria and papillary muscles with EGTA abolished the previously observed differences in contractile force, denoting a lack of effect of diet on the contractile elements of the tissues. There is no clear explanation, however, for the severe drop in the maximum tension developed by skinned left atria in comparison to that seen in the electrically stimulated intact tissues. Kentish and Jewell (1984) noted that thick trabeculae from rat heart also exhibited low contractile tension, which they believed due to a failure of Ca^{2+} to reach all of the myofibrils. It is possible that, despite the thin walls of rat atria, a similar phenomenon was occurring. Alternatively, atria may display greater resistance to the EGTA treatment on account of the different structure of the atrial myocyte.

It is also unclear why papillary muscles from the CAN- group maintained a maximum tension similar to that manifested by intact tissues, while it became considerably less in skinned papillary muscles from SKF- and LIN- groups. It seems unlikely that EGTA exerted a differential effect on tissues of the different dietary groups as physical dimensions were the same. A preferred interpretation is that membrane regulated differences in contractility were abolished by EGTA treatment revealing a uniformity of Ca^{2+} sensitivity at the level of the myofibrillar protein in all diet groups.

Miller (1979a, 1979b) has criticized the use of EGTA to chemically skin cardiac tissue. Using frog heart, he and his co-workers found extremely long-lasting action potentials (and thus long periods of membrane depolarization) and increased sensitivity to bath calcium ion in EGTA-treated tissues (Miller and Moisescu, 1976; Miller and Morchen, 1978). The latter finding was attributed to the Na^{+} - Ca^{2+} exchange mechanism. Miller concluded that his frog preparation was not skinned and that a decreased resting potential and sensitivity to bath calcium are not sufficient criteria that a preparation is skinned. He also speculated that EGTA treatment of mammalian tissue might not result in a functionally skinned fibre. Specific rebuttals to Miller's criticisms have been offered for the use of EGTA to skin mammalian cardiac muscle (Winegrad, 1979). Morphological studies as well as physiological tests (i.e. sensitivity of the preparations to Mg^{2+}-ATP removal (McClellan and Winegrad, 1978), together with the observation of Fabiato (1981) that solutions containing EGTA and millimolar Mg^{2+} caused disruption of the surface membranes of single cardiac cells argue strongly for the adequacy of EGTA skinning in mammalian preparations. Further studies by Miller et al. (1985), using rat ventricular trabeculae, showed that lanthanum ions failed to penetrate the sarcolemma of EGTA treated muscles, although they entered Triton X-100 treated tissues. The contractile time courses were consistently slower with EGTA treated tissue, although the Ca^{2+}-sensitivity was identical to saponin or Triton treated tissue (Miller and Smith, 1985). Kentish and Jewell (1984) concurred in the view that while Ca^{2+} permeability was increased in EGTA treated muscles, some degree of selective permeability was retained and the Ca^{2+} environment of the myofibrils was inadequately controlled. Nonetheless, the consensus is that, ignoring the slower time course of the response, EGTA treated tissues will respond to external calcium concentrations in a manner quantitatively comparable to other means of chemical skinning.

5.3.3 The Contractile Response to Bay K8644

Calcium channels play an important role in excitation-contraction coupling in cardiac muscle. Agents that block these channels depress cardiac contractility. The most potent and specific of the Ca^{2+} channel blockers are the 1,4-dihydropyridines, typified by nifedipine (Vater et al., 1972). Recently, a nifedipine derivative that acts as positive inotropic agent was reported (Schramm et al., 1983a, 1983b). The drug, Bay K8644 is considered to be a calcium channel agonist (Towart and Schramm, 1984): in cardiac cells it appears to enhance calcium influx (Wahler and Sperelakis, 1984). Single cardiac transmembrane Ca^{2+} channels have three modes of gating behavior in the absence of drugs, expressed as current records with brief openings (mode 1), with no opening because of channel unavailability (mode 0 or null mode) and with long-lasting openings and very brief closings that appear only rarely (mode 2) (Hess et al., 1984). The dihydropyridine Ca^{2+} agonist Bay K8644 enhances Ca channel current by promoting the prolonged open state (mode 2) (Kokubun and Reuter, 1984; Ochi et al., 1984). Recently, it was shown that, unlike β-agonists, Bay K8644 positive inotropic action does not involve cAMP as a second messenger (Bohm et al., 1985). It is not clear why the differing responses of atria from the dietary groups of tissues to BAY K8644 at 0.75 mM Ca^{2+} do occur (section 4.5.5.) (Fig. 16).

However, it is possible that the diet induced changes in membrane lipid composition have changed channel kinetics to cause more Ca^{2+} channels naturally to be in mode 2 at any one time in tissues from

SKF-group, and therefore fewer would be available to be unnaturally restrained in this mode of prolonged open state. Thus although SKF tissues had a higher intrinsic contractility, their potential for positive inotropic stimulation by BAY K8644 was less. Also, Ca^{2+} channels in tissues from the CAN- group might have had a greater individual conductance in response to Bay K8644, and thus a greater triggering effect on Ca^{2+} release from intracellular sites resulting in a higher tension development. There has been considerable speculation that alteration in membrane composition could affect ion channel conductance (Katz and Messineo, 1981a; Spector and Yorek, 1985). However, no definitive evidence has yet been provided that ionic current through any natural channel is altered by changes of the type postulated in the present experiments. It remains an item on which definitive evidence is lacking. In contrast to atria, papillary muscles from SKF-group displayed the greatest increase in tension as well as the greatest initial tension (Fig. 17). The other three dietary groups displayed very little response to BAY K8644 which contrasted with their response to increases in external Ca^{2+} concentration (Fig. 4). The reason why this should be so is not obvious, although external Ca^{2+} concentrations affect Ca^{2+} binding as well as current through channels (see 5.3.2). It ought not to be surprising that the response to Bay K 8644 differs from that to Ca^{2+} alone, and may indicate a mechanism for the effect of dietary lipids in influencing contractility.

Associated binding studies using ^{3}H-nitrendipine have indicated no difference in either K_D or B_{max} in myocytes from animals of different diet groups (Dryden et al., 1985). Thus the affinity displayed by a dihydropyridine antagonist for the receptor, and the total number of binding sites appear to be the same in hearts from animals of each group. If this is so, then the differences in response must lie at the level of calcium channel function, i.e. differences in the kinetics of change from the various states of the calcium channel, or differences in individual channel conductance. The differences in response between atrial and ventricular tissue may well reflect differing dependence on the inward Ca^{2+} current in the production of overall tension, particularly in the rat (Fabiato and Fabiato, 1978a; Vassalle, 1979).

5.4 DIETARY LIPIDS AND THE SUSCEPTIBILITY OF CARDIAC TISSUES TO DYSRHYTHMIAS

The results obtained in this study clearly indicate a dietary-lipid influence on the genesis of dysrhythmia in isolated cardiac tissues. Atria and papillary muscles from CAN-group were least susceptible to the dysrhythmogenic effect of Ca^{2+} and isoprenaline.

Dysrhythmias result from abnormalities of impulse initiation or impulse conduction or a combination of both (Hoffman, 1966). In isolated heart tissues dysrhythmia can be easily induced with a high-Ca^{2+} containing bathing solution (Fozzard, 1983). The Ca^{2+} induced increase in contractility can render the isolated tissue hypoxic, as the rate of oxygen utilization exceeds the rate of oxygen diffusion from the bathing medium into the tissue. Both Ca^{2+} and hypoxia will then induce a transient afterpotential (also called an oscillatory afterpotential) that generates spontaneous pacemaker activity (Fozzard, 1983; Wit and Rosen, 1983). Although not without its sceptics, a direct relationship appears to exist between developed contractility in isolated tissues and their susceptibility to dysrhythmia. Atria and papillary muscles from CAN- group displayed both the lowest contractility and the lowest incidence of dysrhythmia at the higher concentrations of Ca^{2+}. The opposite was observed in SKF-group which in itself was not different from the SFO-group, thus contrasting with the findings of Charnock et al. (1985b) after six months of dietary augmentation.

The incidence of dysrhythmic response to isoprenaline was greater than that induced by Ca^{2+}. Tissues from the CAN group continued to show the lowest incidence, but the most sensitive group was LIN rather than SKF. The mechanism of induction of dysrhythmia has long been recognised, yet surprisingly little studied. Recent evidence has suggested that natural catecholamines are not obligatory mediators of dysrhythmia, and that ischemia, or at least hypoxia is the primary stimulus (Daugherty et al., 1986; Szekeres et al. 1986). Adrenaline, in anesthetized cats, caused dysrhythmia that could not be abolished by β-adrenoceptor blockade, although the possibility of an α-adrenoceptor action was not excluded in these experiments (Korczyn and Teplitsky, 1984). As isoprenaline was used in the present experiments, any α-adrenoceptor mediated effect was excluded. However isoprenaline (and adrenaline) in addition to enhanced Ca^{2+} entry during the action potential (Reuter, 1974) caused an acceleration of calcium channel repriming possibly by a mechanism dependent on β-adrenoceptor mediated increases an intracellular cyclic-AMP levels (Sutherland and Rall, 1960; Krebs, 1972; Katz _et_ _al_., 1972; Kirchberger and Wong, 1978; Katz, 1979) and more rapid sequestration of cytosolic Ca^{2+} (Shimoni _et_ _al_., 1984). Such a phenomenon is common in tissues depleted of oxygen. The net effect is that membrane refractoriness is reduced and the tissue is predisposed to re-entrant dysrhythmia. In addition the same authors reported an enhancement of the rate dependent increase in slow inward current. Thus in conditions of hypoxia, where premature ectopic foci arise, the heart is predisposed to respond with a burst of tachycardia. It is doubtful if re-entry pathways exist in the small tissues used in the present work, but the induction of dysrhythmia seems likely to be attributable to hypoxia, as the oxygen demand outstrips the supply available by diffusion from the bath solution. This effect is the greater in the presence of isoprenaline, rather than elevated Ca^{2+} alone, due to the effects of isoprenaline on Ca^{2+} currents.

None of the foregoing, however, includes any role for fatty acids, either in phospholipids, triglycerides or free. However membrane lipid composition can influence the activity of brain adenylate cyclase (Baba *et al*., 1984). If this is a general phenomenon, then heart adenylate cyclase too may be affected, although a preliminary communication by McMurchie (1986) would deny this possibility in the rat.

Catecholamines are well known stimulants of lipolysis of endogenous triglycerides, causing intracellular accumulation of free fatty acids and their metabolites, acyl Co-A and acyl-carnitine (Opie, 1976; Jesmok *et al*., 1977). As indicated in section 5.3.2., significant elevation of free fatty acids can profoundly affect several essential membrane associated functions of the cell. Free fatty acid production in isoprenaline stressed perfused hearts is greatly increased in tissues from SKF animals and much lower in CAN animals (Dryden and Wong - unpublished results) providing experimental substantiation to this latter hypothesis.

The fatty acid composition of cardiac membrane lipids could influence the cardiotoxicity of catecholamines. As previously mentioned (section 1.5) Yates and Dhalla (1975) have suggested that the cardiotoxicity of catecholamines is primarily due to the corresponding adrenochrome (i.e. oxidation product of the catecholamine). They observed, in isolated rat hearts perfused with oxidized isoprenaline, ultrastructural changes and impairment of the functional capacity of cardiac muscle. The same investigators suggest that adrenochromes could in turn stimulate microsomal peroxidation or oxygenation of the polyene fatty acids to various fatty acid derivatives which could affect the cardiac cell adversely.

5.4.1 Influence of Dietary Lipids on Mortality Following Coronary Artery Ligation

The results of the present study demonstrate an influence of dietary lipids on overall mortality following coronary artery ligation (CAL) in anesthetized rats. Animals fed the CAN diet sustained the lowest incidence of mortality in comparison to the other three groups, although the difference was only statistically significant between CAN and SKF fed animals. However, a distinction should be drawn between the causes of death, as those are quite separate and possibly unrelated. When fibrillation alone is considered as the cause of death, there was little difference in the incidence of mortality in CAN and SFO groups, and although the incidence in LIN and SKF was higher, this difference was not significant. It must be admitted that a larger sample size might have provided sufficient numbers in each category of death for existing differences to assume statistical significance. However, the present sample size of ten provided insufficient discrimination. This result agrees with the study reported by Logan et al. (1977) that failed to demonstrate any difference in fatal ventricular dysrhythmias in rats fed richly polyunsaturated safflower oil or more saturated beef fat supplements. It is, however, in direct contrast to the observations of Lepran et al. (1981) and of McLennan et al. (1985). The former work found that rats fed a diet supplemented with sunflower oil offered greater protection from fatal dysrhythmias than standard rat diet. These two diets were not isocaloric, and a close comparison with the present work is difficult. The same cannot be said for the diets used by McLennan et al. (1985). There the two diets (SKF and SFO) were rigorously controlled and isocaloric, almost identical to those used in this study. However some differences in methodology exist that might serve to explain the apparent contradiction in results. The rats used in the present work were much younger at the onset of feeding, and fed for a much shorter period of time, whereas McLennan et al. (1985) used older rats (80d) at the onset of feeding, and continued the feeding for a considerably longer period of time (7 months and 20 months). Significant differences in dysrhythmia score and ventricular extra beats were seen only after 20 months of feeding. This plus other results (Charnock et al., 1985b) led to the suggestion that age plays a role in determining myocardial pathogenicity. Thus the much younger age of the animals used in the present study may have protected them from ventricular fibrillation. Secondly, the degree of ischemia imposed in the present experiments was more global than in McLennan's work, as the ligature was placed higher on the main left coronary artery (Personal communication to W.F. Dryden). Therefore there may be no direct conflict in the results of these two studies.

Cardiogenic shock contributed substantially to the overall incidence of mortality in the present study particularly in the SFO-group. It has not been documented in other reports, but the minor differences in the degree of ischemia stemming from the positioning of the ligature may dictate the nature of the overall cardiovascular response. In the present experiments, the response was all-or-none, and no quantification of the response itself was possible, in contrast to the previously quoted reports.

It is difficult to speculate on why the relative youth of the animals used in the present experiment (and those of Logan et al., 1977, and the 7 month fed animals of McLennan et al., 1985) protected them from the dysrhythmia seen in older animals (McLennan's 20 month fed group) particularly those fed SKF. Analysis of fatty acids present in total ventricular phospholipids reveals progressive changes over a 16 month period of time, but only in the SFO fed animals (Charnock et al., 1983; Charnock et al., 1985a). The fall in $\Sigma\omega$-6 accounted for largely by a depletion of linoleic acid (C18:2,ω-6) present and in the rise in $\Sigma\omega$-3, as docosahexaenoic acid (C22:6,ω-3) proportions increase, indicate a change with time in the fatty acid profile of the SFO group towards that typical of the SKF group, which remained relatively constant throughout.

Although the differences in fatty acid profile remained significant among these groups after 18 months of feeding, this does not correlate with the increase in the susceptibility of the SKF-group to ischemic dysrhythmia at that age. Overall membrane composition alone, and the attendant physical properties cannot explain the observations. Other membrane systems which may involve fatty acids, must play a role in the age dependent responses to ischemia. Cyclic-AMP is known to increase in the ischemic zone of the myocardium prior to the onset of ventricular fibrillation (Podzuweit et al., 1978) and the fibrillation itself has been suggested to be attributable to the excessive trans-sarcolemmal inflow of calcium as a result of elevated cyclic-AMP levels (Podzuweit et al., 1980). As mentioned previously, the activity of the enzyme, adenylate cyclase has been considered susceptible to changes in fatty acid composition in the membrane. However, McMurchie (1986) found no effect of dietary fat on adenylate cyclase activity in the rat.

In contrast to hypoxia alone, the reduction of tissue perfusion in ischemia results in retention of metabolites that may be of primary importance in the genesis of electrophysiological alterations underlying malignant ventricular dysrhythmias (Corr and Sobel, 1982). Two groups of metabolites that have been implicated strongly in the mediation of ischemia-induced dysrhythmias in swine and rabbit, at least, are lysophosphoglycerides and long chain acyl carnitines (Liedtke et al., 1978; Sobel et al., 1978). Both types of compounds, along with free fatty acids, are amphiphiles, capable of causing alterations in sarcolemmal molecular dynamics by insertion or intercalation in the phospholipid bilayer. Such an action could contribute to the electrophysiological and biochemical sequelae of myocardial ischemia in dogs (Fink and Gross, 1984). It is not known at present if a relation exists between the prevalence of certain fatty acids in membrane phospholipids and a greater effect of these compounds in the induction of fatal dysrhythmias following ischemia.

The higher incidence of cardiogenic shock in SFO-group is an important observation that requires comment. Occlusion of a coronary artery stimulates receptive endings of both myelinated and unmyelinated vagal afferent fibres from the heart (Brown, 1966; Recordati et al., 1971; Oberg and Thoren, 1973) to bring about a severe drop in blood pressure (Constantin, 1963; Pelletier, 1979). Whether dietary lipid manipulation could influence such a reflex mechanism has yet to be determined.

Recently, however, a role has been suggested for certain eicosanoids (thromboxane A_2, leukotriene C_4 and leukotriene D_4) in the pathogenesis of ischemia-induced shock (Lefer, 1985). These mediators are believed to exert cardiac depression secondary to their potent coronary constrictor effects (Ribeiro and Lefer, 1983; Roth et al., 1984). Thus the increased availability of linoleic acid in cell membrane phospholipids of SFO-group, could have led to an increased release of these mediators from within the hypoxic myocardium with the development of the resultant shock state.

It is unlikely that any altered incidence of thrombosis contributed to the dietary effects observed, since SKF diet has been found to increase the risk of thrombosis in rats while the opposite was found for SFO-diet (McIntosh et al., 1985), yet in the present work, overall mortality was about the same in both groups and even slightly higher in SFO-group.

5.5 THE POSSIBLE ROLE OF THE EICOSANOIDS IN MEDIATING THE DIETARY LIPID INFLUENCES ON THE HEART

That eicosanoids are involved in myocardial contractile function is suggested by the ability of indomethacin to abolish the differences in responses to Ca^{2+} of left atria and papillary muscles from the four dietary groups. Also, but with the exception of papillary muscles from CAN-group, indomethacin produced generalized reduction in contractility of tissues across all dietary groups.

The pattern of dysrhythmia initially observed with calcium and isoprenaline was altered by the presence of indomethacin. Left atria from SFO-group failed to develop dysrhythmia, and this was significantly different from the other three groups when challenged by isoprenaline. Also, the percentage of papillary muscles from SFO-group that manifested dysrhythmia to either calcium or isoprenaline, became noticeably lower in the presence of indomethacin.

Prostacyclin (PGI_2) was observed to exert a positive inotropic effect on guinea pig isolated heart tissues (Fassina et al., 1983). This effect is believed to result from enhancement of Ca^{2+} transmembrane fluxes (Fassina et al., 1984) and Ca^{2+} release from the sarcoplasmic reticulum (Salvatori et al., 1984). More recently, Couttenye et al. (1985) reported that PGI_2, PGE_1 and PGE_2 do not have a direct influence on the contractility of the rat myocardium. This report added to the confusion surrounding the role of the eicosanoids in cardiac function.

Prostaglandins were thought to be endogenous antidysrhythmic agents (Forster, 1976). Such a belief was later proved to be unfounded as the effects of different prostaglandins on dysrhythmia were shown to vary with animal species, dose and route of administration of the eicosanoids (Coker, 1982). Prostacyclin is a good example; it has been reported to be antidysrhythmic (Coker and Parratt, 1981) and dysrhythmogenic (Au et al., 1979) in rats, to a variably dysrhythmogenic action in cats, depending on dosage (Dix et al., 1979), and to be antidysrhythmic in dogs (Ribeiro et al., 1981; Starnes et al., 1981). Even thromboxane A_2 which has been constantly blamed for its dysrhythmogenic action during acute myocardial ischemia (Coker et al., 1981), was recently shown not to contribute to dysrhythmogenesis during evolving myocardial infarction (Kramer et al., 1985).

It has been suggested that the increase in $\omega6/\omega3$ ratio in rat hearts fed linoleic acid-rich diet leads to an increased availability of substrate for prostaglandin production (Kramer, 1980; Lepran et al., 1981; Charnock et al., 1983). The results of the present work disagree with such a hypothesis as it is apparent that the differences in myocardial function observed cannot be ascribed to any single major change in the fatty acid profile. Our findings are supported by the results obtained by Abeywardena and Charnock (1985), which showed a difference in the production of prostacyclin by aortae from tuna fish oil- and SKF-fed rats despite a similar $\omega6/\omega3$ ratio. Thus, it appears that complex changes in membrane fatty acid composition can result in complex changes in the balance of interactions between fatty acids and the respective enzymes which may influence both quantity and type of eicosanoid released.

It is important to note that indomethacin was reported to inhibit the enzyme phospholipase A_2, and such effect can alter the production of prostaglandins as well as leukotrienes (Kaplan et al., 1978; Shakir et al., 1985). Also, indomethacin was reported to inhibit both adrenochrome formation and polyene fatty acid oxygenation (Gudbjarnason and Hallgrimson, 1979). Thus, abolition of some of the dietary differences by indomethacin is only suggestive and not a proof of a role of eicosanoids in the genesis of these differences.

5.6 A ROLE FOR FREE RADICALS

Oxygen-derived free radicals have been proposed as general mediators of tissue injury in a variety of disease states (Del Maestro, 1980). Reactive species including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH°) may be generated by a number of cellular reactions (Fridovich, 1978). It has been shown that xanthine oxidase can act on xanthine to produce oxygen free radicals by the following reactions (Beauchamp and Fridovich, 1970):

$$\text{xanthine} + O_2 \xrightarrow{\text{xanthine oxidase}} \text{urate} + O_2^-$$

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

The second reaction is spontaneous or it may be catalyzed by superoxide dismutase (SOD) (Hess et al., 1981). Superoxide anion radical and H_2O_2

can then interact to generate free hydroxyl radical (OH°) according to the following reaction (Haber and Weiss, 1934; McCord and Fridovich, 1968):

$$O_2^- + H_2O_2 \longrightarrow O_2 + OH^- + OH^\circ$$

The results of the present study clearly show a protective effect by either allopurinol (xanthine oxidase inhibition) or mannitol (OH° radical scavenger) against the dysrhythmogenic effect of isoprenaline on left atria from all dietary groups. However, a conceptual difficulty arises in that xanthine oxidase is an intracellular enzyme, requiring O_2^- to lead to the generation of OH° within the cytoplasm. The O_2^- anion is unlikely to leave the cell easily, and OH° is highly reactive, with a mean free path of less than 1 μm. It is inconceivable that such a radical can permeate the cell membrane to have an extracellular action.

Mannitol diffuses into the cell only slowly, if at all (Elbrink - personal communication), and its main site of action is likely to be the extracellular medium. Preliminary experiments, not reported in Results, in which SOD was placed in the bathing fluid failed to prevent the onset of dysrhythmia, and when exogenous purine and xanthine oxidase were used, no dysrhythmia was generated. Resolution of the paradox lies in the observation that at the concentrations used in the present work, allopurinol like other purines, though only a mild scavenger of the superoxide anion (Peterson et al., 1986) can function as an OH° scavenger in conditions of mild OH° generation (Hochstein - personal communication). Thus, the generation of OH° radicals at the cell surface, as in eicosanoid synthesis, may represent the means whereby free radicals mediate the onset of dysrhythmia in these cells. These findings suggest a main role for the OH° radicals, which is further supported by the findings of Burton _et al_. (1984) that showed a more extensive damage in cardiac septa when perfused with solutions generating OH° radicals, than when superoxide-generating solutions were used. Papillary muscles, on the other hand, were not significantly protected by attempts to scavenge free radicals, regardless of the dietary group. The reason behind such a variation in response between atria and papillary muscles is not known at the present time.

It is important to notice that the use of allopurinol and mannitol resulted in changing the pattern of dysrhythmia induced by isoprenaline alone, previously observed in both types of tissue. The presence of either of these compounds caused a lower incidence of dysrhythmia in left atria from LIN- and SFO-groups while tissues from SKF-group sustained the highest incidence. Papillary muscles showed a similar disposition, but only in the presence of mannitol. Such results may indicate an influence of dietary lipids on the generation of oxygen-derived free radicals. The interrelationship between membrane polyunsaturated fatty acids and the oxidized products of catecholamines has been discussed previously (section 5.4).

Recently proposed mediators of myocardial ischemia involve the generation of oxygen-derived free radicals (Wexler and McMurtry, 1981;

Manning *et al.*, 1984; Chambers *et al.*, 1985). Any free radicals generated during myocardial ischemia would readily interact with the unsaturated fatty acids of membrane phospholipids (Rao *et al.*, 1983a). This interaction may then generate new radicals in a chain reaction which might lead to increase membrane permeability (Singal *et al.*, 1982), and eventually result in extensive cellular damage at the sarcolemma, sarcoplasmic reticulum, and mitochondria (Jennings and Ganote, 1974; McCallister *et al.*, 1978; Hess *et al.*, 1981; Burton *et al.*, 1984). However, the precise role of membrane phospholipids and the exact types of damage that result from myocardial exposure to free radicals remains to be established.

5.7 EFFECTS OF DIETARY LIPIDS ON SARCOPLASMIC RETICULUM FUNCTION

The S.R. is a relatively simple membrane, as its primary, if not exclusive, function is to regulate cytosolic Ca^{2+} concentration (Katz, 1984). As has already been pointed out, the rat myocardial contractile function depends to a significant extent on the amount of Ca^{2+} stored within, and released from the S.R. (Bers *et al.*, 1981; Bers, 1985). The process by which Ca^{2+} is transported into the S.R. is an active one in which Ca^{2+} is moved against its electrochemical gradient by an ion pump (Ca^{2+}-ATPase) that involves expenditure of energy (Tada and Inui, 1983). However, the nature of the process by which Ca^{2+} is released from this structure in the intact muscle is poorly understood. There is a general agreement that it is a passive downhill process (Fabiato and Fabiato, 1977; Katz, 1984); induced by depolarization, inositol triphosphate (Vergara et al., 1985), or Ca^{2+}, the last suggestion having the greatest support (Winegrad, 1982). Although Ca^{2+} release from the S.R. is most directly related to contractility of the whole cell (Katz, 1984) its measurement requires unusual sophistication. Manual skinning of fibres can be performed, after the acquisition of skill, or a luminescent or fluorescent material such as aequorin can be injected into the cell to emit light during the few milliseconds that free Ca^{2+} concentration in the cytosol is raised following excitation. As the primary question to be answered concerned an influence of dietary lipid variation on the sarcoplasmic reticulum, it was decided to investigate the phenomenon of calcium uptake rather than release, and so avoid the technical difficulties that the latter option demanded.

Results obtained show a clear effect of lipid-supplemented diets on the rate of uptake of Ca^{2+} by cardiac S.R. vesicles. Although it is not suggested that contractile tension is dependent on the rate of Ca^{2+} uptake by the S.R., the groups were arranged in rank order similar to that observed in the Ca^{2+}-twitch tension study. Left atria showed an inverse relationship between the rate of Ca^{2+} uptake by S.R. and the magnitude of the contractile response to Ca^{2+}. In ventricles, however, the relationship was a direct one. Assuming that the fraction obtained from atria and ventricles represented the same subcellular component purified to the same extent, these results appear to indicate either differences in the response of S.R. membranes to varied fatty acid supply, or to indicate a fundamentally different relationship between the Ca^{2+}-uptake process, and surrounding S.R. membrane phospholipids in atria and ventricles. No specific information on the first point is available although Gibson _et al_. (1984) found no significant difference in the fatty acid composition of phospholipids in heart microsomes (analogues to the ventricular S.R. used in this work) and that reported in whole ventricular phospholipids (Charnock _et al_., 1983). If a similar relationship exists in atria, then it is difficult to conceive of a mechanism that would account for the reversal of the rank order of Ca^{2+} uptake, despite the small differences in the fatty acid composition of total phospholipids from the two regions of the heart. On the other hand, the contractile, and relaxation parameters of atria and ventricular muscle differ considerably, and a fundamentally different relationship between the Ca^{2+}-ATPases of atrial and ventricular S.R. and the surrounding phospholipids, though surprising, is not impossible. Most research on S.R. Ca^{2+}-ATPase has used ventricular tissue. No data appear to be extant on the enzyme from atrial S.R., with particular reference to its relationship with the surrounding membrane phospholipids. However, it has been shown that C_{18} and C_{20} _cis_-unsaturated free fatty acids markedly inhibit Ca^{2+} uptake by S.R. vesicles derived from ventricles (Cheah, 1981; Katz _et al_., 1982; Munkonge, _et al_., 1985). While no information on the intercalation of free fatty acids in the S.R. membrane preparations used here is available, the possibility exists that dietary preconditioning with free fatty acids, or alteration of phospholipid fatty acids may account for the differences in Ca^{2+} uptake observed.

In addition to the effect on Ca^{2+} uptake by the S.R., dietary lipids influenced the activity of the ATPase enzymes in the same preparations. No correlation, however, appeared to exist between the rate of uptake of Ca^{2+} and the activity of any of the ATPases among the four dietary groups. This can be due to the fact that the present study on ATPases was carried out only on the ATP-hydrolyzing capacity of the enzymes which is thought to be different from their function as ion pumps, and these two modes can be affected differently by dietary lipid manipulation (Tada and Inui, 1983).

The lack of dietary influence on the activity of ventricular S.R. Ca^{2+}-dependent ATPases, reported in the present study, is supported by the findings of Abeywardena et al. (1984). This group showed no difference in the ATPase activity of S.R. Ca^{2+}-ATPase from hearts of rats fed either SFO- or SKF- supplemented diet, despite a demonstrable change in membrane fluidity (decreased in SKF) and a marked alteration in phospholipid fatty acid composition. Another recent study by McMurchie et al. (1983), showed a lack of sensitivity of mitochondrial F_1-ATPase to changes occurring in the composition of the membrane lipids. Both reports suggested that the ATPase systems which completely span the membrane and require a fixed orientation to carry out their function, seem to be protected from or insensitive to changes in the bulk lipid matrix. The present results, however, do not agree with this concept as dietary manipulation did result in changes in the activity of ventricular S.R. Ca^{2+}- independent ATPases and probably in atrial Ca^{2+}-dependent ATPases. This implies that enzymes belonging to such systems may have certain components capable of undergoing lateral diffusion and can therefore be affected by changes in membrane physical properties (Robblee and Clandinin, 1984).

5.8 HISTOLOGICAL CHANGES INDUCED BY DIETARY LIPIDS

The highest incidence of focal heart lesions (5/7; 71%) was observed in left ventricles from rats fed rapeseed oil (erucic acid free). Such finding suggests that erucic acid is not responsible for rapeseed oil induced cardiac lesions, as has been frequently reported by many investigators (Abdellatif and Vles, 1970, 1973; Beare-Rogers et al., 1974; Engfeldt and Brunius, 1975; Vogtmann et al., 1975; Hulan et al., 1976). Both De Wildt and Speijers (1984) and Kramer, Fannworth and Thompson (1985) have also thrown doubt on the conventional incrimination of erucic acid (see section 1.5) in the appearance of focal lesions. The likelihood that the lower contractility in CAN-papillary muscles is caused by such small necrotic lesions affecting so little of the myocardium is difficult to accept as similar lesions were found in one animal from SFO-group without an effect on force development. Also, those animals in CAN-group which did not sustain similar lesions did manifest a lower twitch tension.

The reason for a higher incidence of cardiac lesions in CAN- group is not known at the present time. The observation of Kramer, Farnworth and Thompson (1985) that a reciprocal relationship existed between dietary saturated fatty acids and lesions, and a direct relationship between membrane phospholipid C22,ω-3 polyunsaturated fatty acid content is in complete agreement with the present work and suggests that the lesions form, not as a response to a single toxic constituent, but is related to the ill defined metabolic control that relates the saturated fatty acid supply to the elevated C22,ω3 content of cell membranes (see 5.2). Although hearts from the SKF group had a higher content of C22,ω-3 acids they presumably were protected by the high saturate content of the initial diet. On the other hand, the mild lipidosis seen in all four groups implies that fat deposition is related to quantity rather than class of lipids in the diet.

5.9 CONCLUSION

The results of the present study clearly show that dietary lipid manipulation can lead to alteration in both the fatty acid composition of membrane phospholipids, and the function of the heart in terms of contractility and rhythmicity under conditions of stress. Also, atria were shown to be distinct from ventricles in all responses.

In spite of the fact that the dietary effects were shown to be at the membrane level, the observed differences in contractility and susceptibility to dysrhythmia could not be ascribed to any single major change in membrane fatty acid profile. It is clear that complex changes in membrane fatty acid composition are associated with complex changes in the function of different membrane-bound proteins (channels, enzymes or receptors) with resultant changes in the overall function of the cell. Also, such complex interactions can influence the availability (quantity and type) of certain mediators as the eicosanoids or harmful factors as the free radicals.

The finding that diets exerted a differential effect at the level of the S.R. (Ca^{2+}- uptake and ATPases activity) add to the complexity of the issue, and makes the frequently suggested alteration in eicosanoid production, as the main mechanism behind altered functional responses, an oversimplification of a very complex matter.

Finally, this study shows that the decreased contractile response in tissues from CAN- group cannot be attributed to the presence of focal necrotic lesions, and such lesions may be related to quantity rather than class of lipids in the diet.

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